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THE PERMEABILITY OF NATURAL MEMBRANES

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To

PROFESSOR J. G. DRUMMOND
who first interested us in this field, and
for whose encouragement we are
so greatly indebted

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Note. Readers primarily interested in conclusions can profitably omit reading Chapters II, III and IV

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NOTE

In places it has been necessary to include unpublished results or calculations. Those by H.D. are marked **, those by J.F.D. ††

FOREWORD

By E. NEWTON HARVEY, *Professor of Physiology,
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JUST as chemistry could not have developed without test tubes to hold reacting substances, so organisms could not have evolved without relatively impermeable membranes to surround the cell constituents. This barrier between the inside and the outside, the inner and external world of each living unit, has been and always must be considered one of the fundamental structures of a cell. No one can fail to be impressed with the great difference in properties of living and dead cells. The dead are completely permeable to diffusible substances, while the living retain one material and pass another. This difference, selective permeability, is so marked that it becomes the surest test to distinguish the living from the dead, holding where all other methods fail. It can truly be said of living cells, that by their membranes ye shall know them.

There can be no doubt of the fundamental importance of cell permeability. Several symposia on membranes have been held recently, notably that of the Faraday Society in 1937 and that at Cold Spring Harbor in 1940, but no general books on cell permeability have appeared for over ten years. It was, therefore, with great pleasure that I learned of Drs Danielli and Davson's plan to bring together the existing knowledge in a book dealing with natural membranes. Such an undertaking is no mean task. Cell permeability has passed from the qualitative to the quantitative stage and the detailed data now available would baulk less enthusiastic authors, even in normal times. The compilation will be even more appreciated in that it has been carried out under the stress of war. Cell physiology will be grateful indeed for this summing up of a subject which is destined for rapid development under the stimulus of modern methods of exploring molecular dimensions and molecular arrangement. Viewpoints may differ but the facts remain. These are systematically and logically presented in this timely volume.

AUTHORS' PREFACE

IN this book we endeavour to give a general survey of the field of permeability. We have included materials essential for students of Medicine, Physiology, Biochemistry, Zoology and Botany, and have provided key references so that the literature on any point which it is desired to pursue further may be looked up with a minimum of trouble. We hope this will assist those lecturing on permeability, and will accelerate the disappearance of the many errors which have crept into the literature designed for students.

The last twenty years have seen a steady development of exact measurements of membrane permeability, mainly due to the American schools of Lillie, Lucké and McCutcheon, and Jacobs, but also largely contributed to by the Finnish school of Collander and Bärlund. To-day we may on the one hand say that the experimental side of this field is now mainly quantitative. During the same period physical science has made many advances in the fields of surface chemistry and the structure of liquids and solids. These have provided us with the basic materials for a quantitative theory of permeability. To some extent we have incorporated such a theory in this book, drawing principally on the work of I. Langmuir, W.D. Harkins, N.K. Adam, E.K. Rideal and E.N. Harvey when dealing with membrane structure, and on the theory of activated diffusion and on such work as that of Fowler & Bernal in achieving a description of the process of penetration of a membrane. So that, on the other hand, the theory of permeability has begun to take a quantitative form. We believe that we may now definitely claim that permeability studies have passed beyond the preliminary exploratory stage, and have reached the stage at which quantitative analysis is of dominant importance. This first attempt at such analysis will, we hope, merely be the precursor of a more exact study.

As J. Loeb complained many years ago, obscure or inexplicable phenomena in biology are fashionably brought into the currency of "knowledge" by way of the philosophers' stone "a change in permeability". When to this the more modern elixir of "surface action" is added, night unto night sheweth knowledge. Such speculations serve a useful purpose in giving an apparent

coherence to scattered and isolated observations, so encouraging the collection of more facts. But the sooner superficialities are replaced by a detailed understanding of underlying mechanisms, the better for science. We hope that this book will assist in defining what can, and what cannot, be done by the cell membrane, by "surface action" and by "changes of permeability". We have not included a chapter on monolayers, etc., since the books of Adam and Rideal are a much more adequate introduction to the necessary fundamentals of surface chemistry than we could hope to provide.

We wish particularly to thank the many friends who have helped us, both by friendly criticism and by providing us with laboratory facilities to carry out our studies—Professor J. C. Drummond, Professor A. V. Hill, Professor C. Lovatt Evans, Professor Sir Frederick Gowland Hopkins, and Dr J. Needham in England; and Professor E. N. Harvey, Professor M. H. Jacobs and Dr E. Ponder in America. We have also, at various times, been assisted by the advice and criticism of Professor J. D. Bernal, Dr A. C. Burton, Professor R. Collander, Dr L. H. N. Cooper, Dr S. L. Cowan, Dr H. J. Curtis, Dr C. Goodeve, Professor R. Höber, Professor D. Keilin, Dr M. Maizels, Dr D. Mazia, Professor W. J. V. Osterhout, Dr A. K. Parpart, Dr J. H. Quastel, Dr F. J. W. Rough-ton, Dr H. Shapiro, Dr J. H. Schulman, Mr F. J. Turton, Dr W. Wilbrandt. No one mentioned here, however, do we wish to burden with responsibility for any views advanced in this book, except where expressly stated.

We are also indebted to Mr J. F. Danielli, Senr., who read the proofs, to Dr A. Neuberger, who read part of the proofs, to Dr W. A. H. Rushton, who read the proofs of Chapter xv, and to Mrs Mary Danielli for assistance with the manuscript, proof and index.

The tardiness of communications, due to the war, has prevented full discussion of some points, so that responsibility for the views advanced in the individual chapters must be mainly borne by one or the other of us.

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CHAPTER I

SIGNIFICANCE OF PERMEABILITY STUDIES

THE distinction between the interior of a cell and the medium which surrounds it is maintained by a membrane about one millionth of a centimetre in thickness. Continued existence of the cell is dependent on the ability of this membrane to permit passage of some substances and prevent that of others. For the more complex organisms other membranes become essential, such as those of the blood capillary, the glomerulus of the kidney, the blood-brain barrier, etc. The study of these various membranes is a basic branch of physiology. From its study information of two kinds is obtainable: (1) knowledge of the structure of the membranes; (2) knowledge of part of the mechanism whereby living bodies maintain their composition and ability to function. By measuring the permeability of cells to various substances we obtain information which enables us to predict the relative rates at which various types of molecules will penetrate into cells. Then, confronted with, say, a group of drugs, we should be able to predict that some will penetrate rapidly into cells and others very slowly, if at all. Thus we are able to define an important group of drugs whose action must be on the surface of cells, since they do not penetrate sufficiently rapidly to produce their action on the interior of cells. A similar problem arises with metabolites. It will be our task here to analyse experimental observations of permeability so that a straightforward answer may be given to such practical questions. In doing so we find that substances fall roughly into two main groups: (a) substances which diffuse according to the laws of thermodynamics, only from a region of higher to one of lower concentration,* so that in the final equilibrium condition the substance is in the same concentration on both sides of the membrane; (b) cases where the laws of thermodynamics are apparently broken and molecules accumulate on one side of a membrane, in excess of the amount on the other side.

* To be exact we should write *chemical potential*, not *concentration*. According to the Second Law of Thermodynamics a substance diffuses from a region of higher to a region of lower chemical potential. In biological systems concentration is usually proportional to chemical potential, and thus may be used instead.

As an example of this latter phenomenon may be taken the frog kidney tubule: the fluid entering the tubule from the glomerulus has approximately the same concentration of chloride ion as the blood plasma, but practically all of this chloride ion diffuses across the tubule membrane into the vascular system, against a concentration gradient, so that the urine is practically chloride free. As it is highly improbable that the Second Law of Thermodynamics is actually broken, we at once infer that in such cases the cells concerned supply energy for the transport of molecules. Thus the two groups of molecules are really (*a*) those towards which the behaviour of the cell membrane is passive, and (*b*) those towards which it is active. The former group of substances is much the larger, and the details of the mechanism whereby these substances pass through the cell membrane are now fairly well known. The second, smaller, group of molecules, subject to secretory activity, accumulates in cells by mechanisms which are still almost completely unknown. It is very probable that the power of a cell to accumulate or excrete certain substances and not others, against a concentration gradient, is intimately connected with the structure of the membrane. The accumulation or excretion must, of course, also be connected with the special nature of the cell's metabolic processes, and it is of fundamental importance to determine the nature of this metabolism. The problem of secretion, therefore, presents a problem to the specialist in cell permeability as well as to the specialist in metabolism; the study of the nature of the membrane, running hand in hand with the study of the special nature of the metabolic processes involved in secretory activity, will eventually lead to the solution of many of the problems of secretion and growth.

The connection between changes in cell membrane permeability and the function of the cell is not clearly defined; however, some interesting correlations have been obtained. Definite changes in the rate of penetration of alkalis into marine eggs on fertilisation were established as long ago as 1911 by E.N. Harvey, and since then changes in permeability to water and certain dissolved solutes have also been established by various workers. Cyclical changes in the permeability of the developing egg, correlated with successive divisions, have been claimed to exist by Herlant (1918). O. Warburg, in a series of investigations on the oxygen consumption of cells, has shown that changes in the membrane are

often correlated with large changes in the oxygen consumption (*cide* Warburg, 1910). Again, it has been shown that narcotic substances, i.e. substances which depress the metabolic activity of cells, will, in the same concentrations in which they exhibit this narcotic effect, also depress the permeability of certain cells to penetrating substances. But narcotic substances may also increase cell permeability and it has yet to be shown that there is, in fact, any direct functional relationship between permeability and narcotic action. Then again, it is believed that the contraction of a muscle fibre is a response to a transient increase in permeability of the fibre plasma membrane. But in most of these examples, as in the others which could be given, experimental evidence is slender and the theoretical basis tenuous. Consequently, in the following chapters, we shall deal mainly with experimental results and with membrane structure, while relationships with other physiological fields will be only roughly indicated.

Now let us consider the precise meaning of the phrase "permeability of a membrane". We can express this quantitatively as the amount of substance in gram mols penetrating in a given time. Obviously the area of the membrane will be important and also the concentration difference across the membrane. The unit of area most convenient for biological systems is the square micron, so that by "the permeability of a membrane to a substance" we refer to the net number of gram mols of that substance diffusing through an area of one square micron of membrane in one second, per gram mol per litre concentration difference across the membrane. No reference is made to the thickness of the membrane in defining its permeability, but on the other hand the temperature has to be defined. Thus the prime variables which must be defined in experimental work are (1) time, (2) membrane area, (3) concentration difference across the membrane, (4) temperature, and (5) pressure.

The area of the membrane available for diffusion is a quantity which in all permeability studies is equated to the geometrical area of the cell membrane, determined by microscopic examination: this is done simply from lack of information regarding the detailed structure of most, if not all, cell membranes. If the diffusion of the substance being studied is possible through all parts of the membrane, and if there are no submicroscopic convolutions of the surface, then this procedure is justified. Suppose,

number in any given sample will represent the mean behaviour of the erythrocytes of a given animal with a considerable degree of accuracy.

- d. It is a more robust cell than are e.g. marine eggs, and it may be centrifuged at high speeds without serious damage (there are exceptions to this).
- e. For moderate volume changes it increases in volume without increasing in area, owing to its special shape, so that the equations describing the rates of penetration of a solute or water into the cell are much simplified.

For these reasons a great deal of work has been done on this cell, and furthermore that which has been done is generally of a more quantitative nature than many studies on other cells.

Methods of Measurement of Permeability. The various methods used may be separated into three groups: (1) those cases in which the amount of penetration is estimated by a direct chemical procedure; (2) cases in which volume changes are studied by some physical measurement; (3) estimation of rates of penetration by spectroscopic methods. Of these groups, chemical methods only are wholly reliable, though usually tedious. Physical methods, when used with discrimination, have many advantages technically, but are more apt to give misleading results.

The Chemical Method. This is a direct method, which, provided the chemical estimation is specific, is the one to be chosen whenever practicable. The substance to be studied may be added directly to the whole blood, and after definite times samples of the blood may be removed, centrifuged, and either the cells or supernatant fluid analysed. Alternatively, samples of the cells may be added directly to a solution of the penetrating substance in Ringer or serum and later centrifuged down and the cells or fluid analysed.

Remarks. Suppose the substance is added to whole blood, and the serum is to be analysed. The presence of the penetrating substance causes a continuous change in the volume of the cells, and therefore a change in the concentration of the substance in the plasma *due to water shifts*, apart from changes caused by penetration into the cells. These may be accounted for by haematocrite determinations (see p. 7), but this introduces a fairly large error. This difficulty may be overcome by estimating

the concentration inside the cells instead of that in the serum, provided all the cells in a given sample of the original blood are taken (Davson, 1934). Here, however, another objection is introduced; since it is impossible to exclude the plasma completely from the cells, a considerable error is introduced by the inclusion of quantities of serum, which contains a high concentration of the penetrating substance, with the cells. With slowly penetrating substances, however, this difficulty may be overcome by washing the cells with a Ringer's solution after the given times have elapsed. Davson has applied the method successfully to the penetration of potassium into the cat cell; in this case, if cells are suspended in isotonic KCl, potassium penetrates the cells to cause a rise in the concentration of potassium from 30 to about 100 mgm. per 100 ml. H_2O in 1 hour. The interstitial KCl may then be removed by washing with ice-cold NaCl.

Other objections are the inaccuracy in the measurement of the times of the actual suspension of the cells, owing to the uncertainty in the evaluation of the actual time of suspension during centrifuging (this proves not to be very important), and also the necessity for centrifuging which may produce changes of itself (Davson & Danielli, 1938). The method is, of course, not applicable to rapidly penetrating substances (see, however, Dirken & Mook, 1931).

Cryoscopic measurements of the changes in concentration of the serum may be made in place of the chemical determinations (Hedin, 1897); this, however, considerably reduces the accuracy of the method.

The Method of Measuring Volume Changes. If cells are placed in an isotonic Ringer's solution to which the penetrating substance is added, they will first shrink, due to loss of water, and subsequently swell, due to gain of water, as the substance enters the cells. The rate at which the swelling occurs will give a quantitative estimate of the rate of penetration of the substance considered. The measurement of the volume changes may be made by use of the haematocrite (e.g. Mond & Hoffmann, 1928), i.e. centrifuging a sample in a graduated tube and measuring the height of the column of cells, or by diffraction methods if the cells are first converted to spheres (Ponder, 1934), or by the dispersion of light measured with a photo-electric cell (Ørskov, 1933). The haematocrite is not a reliable instrument (Ponder, 1934); but the dispersion

of light seems to be a good index to the relative volume changes of the cells.

Haemolysis Method. It has long been known that if a cell is placed in a solution of a penetrating non-electrolyte, it swells until it bursts, i.e. haemolyses; the rate of haemolysis may be used as an index to the rate of penetration. This method was first used by Griyns (1896) and is now widely used for non-electrolyte studies. The degree of haemolysis is usually measured by an optical method based on the change in the degree of scattering of light by a suspension of cells, after the bursting of the latter. Thus if we mix one drop of blood with 25 ml. of an isotonic solution of a penetrating non-electrolyte, e.g. 0.33 *M* glycerol, in a boiling tube, it is found that on holding the tube in front of a luminous filament, or other bright body, the latter cannot at first be seen: as haemolysis proceeds the suspension becomes less opaque, until at the stage when about 75% haemolysis has occurred the filament becomes distinctly visible.

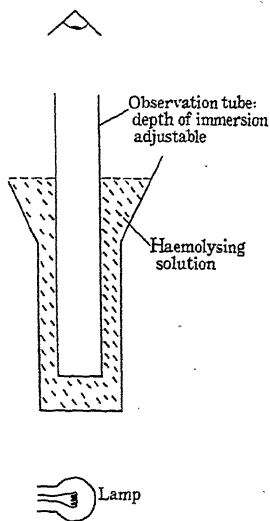


FIG. 1. Diagram of apparatus used by Jacobs for determining percentage of haemolysis.

This principle has been utilised by Jacobs (1930), who describes a method in which the minimum depth of a suspension of erythrocytes necessary to prevent perception of a glowing filament is observed. The filament is at standard brightness. As haemolysis proceeds, the light-transmitting powers of the suspension improve, and the minimum depth increases. The time course of the change in depth is recorded on a kymograph. Alternatively, the instrument may be set so that when the filament is just visible a known degree of haemolysis has occurred; the time required for this to occur is used as an index to the rate of penetration of the non-electrolyte (Fig. 1).

We have already seen that a given erythrocyte can swell up to a certain size, and will then haemolyse. But not all erythrocytes haemolyse at the same tonicity. Fig. 2 shows the percentage of

haemolysis of ox erythrocytes after 1 hour in NaCl of various concentrations. Jacobs (1932) has worked out the relationships between the permeability to water, the osmotic concentrations inside and outside the cell, and the time required to reach a given degree of haemolysis (see Chapter iv). From this the permeability constant to water may be calculated by observing the time required to reach, say, 75% haemolysis in either water or hypotonic solutions of non-penetrating substances, such as NaCl or sucrose.

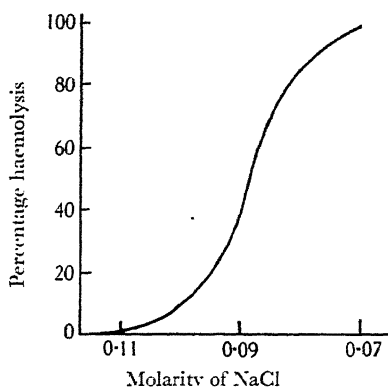


FIG. 2. Percentage haemolysis of ox erythrocytes, plotted against tonicity (after Jacobs & Parpart, 1931).

Where substances other than water are concerned the situation is more complicated, since the penetrating solute and water are penetrating simultaneously. Jacobs (1934) has discussed this in some detail, and has evolved a system whereby, if the value of the permeability, P_w , to water is known, it is sufficient to determine t_s/t_w , the ratio of the time taken in reaching a given percentage of haemolysis in isotonic solute to that taken in water. Then the corresponding value of P_s/P_w is read off from a graph, and, since P_w is known, P_s , the permeability to the solute, can be calculated.

However, most workers seem to have found it sufficient to use the times for the cells to reach a given degree of haemolysis, say 75%, as a measure of the relative rates of penetration of different substances. This is only accurate for times of haemolysis in excess of 1 min.

Remarks. The method involves a decrease in the electrolyte content of the medium surrounding the cell, since, if the concentration of electrolytes is equal on both sides of the membrane (or greater outside than the concentration at which hypotonic haemolysis begins), no haemolysis occurs when a substance penetrates from a suspension medium. Thus Höber & Ørskov (1933) use a concentration of 0.1 % NaCl and Jacobs & Parpart (e.g. 1931) simply add one drop of blood to 25 ml. of non-electrolyte solution. Jacobs has found that under these conditions it is difficult to control the *pH* of the cell suspension, and Davson has found that under these conditions the cells may be abnormal in that they are permeable to cations before haemolysis occurs. Both these factors introduce errors into the calculated permeability constants.

The necessity for distinguishing between a change in the actual rate at which a substance penetrates the cell and a change in the equilibrium conditions between the cell and its environment will be emphasised in Chapter III; in applying an indirect method of measuring permeability such as this, where no chemical determinations whatsoever are carried out, it becomes even more essential to bear this in mind. The point is best illustrated by some work of Jacobs & Parpart (1932), who found that urethanes markedly reduce the amount of haemolysis finally attained in hypotonic salt solutions; a suspension of erythrocytes in 0.08 *M* NaCl which was 75 % haemolysed in 20 sec. required 120 sec. to reach the same degree of haemolysis in 0.08 *M* NaCl + 0.008 *M* phenyl urethane. Now phenyl urethane in 0.008 *M* solution is capable of changing the time for haemolysis by less than 0.1 sec. by direct osmotic effect, i.e. if replaced by an osmotically equivalent amount of NaCl, the time to reach 75 % haemolysis would be changed by less than 0.1 sec. Some authors have therefore concluded that the action of urethane is to decrease the cell permeability to water, and thus increase the time for reaching 75 % haemolysis. But Jacobs & Parpart showed that the effect on permeability is comparatively small, and the major action of urethanes is to change the cell in some way, so that the total amount of water which must enter before haemolysis can take place is markedly increased. To make a correct comparison of the relative rates of penetration of water in the presence of various substances which affect permeability, it is necessary to follow the

time course of the percentage of haemolysis and correct for any changes which occur in the total amount of haemolysis, before calculating relative rates of penetration.

Fragility Changes. By fragility is meant the reciprocal of the dilution of the isotonic medium surrounding the cells required to produce a given degree of haemolysis. This will depend, other things being equal, on the concentration of osmotically active substances in the cell, so that if a substance has penetrated it will increase the fragility. By measuring the dilution of the initial isotonic concentration required to produce a given degree of haemolysis, approximations to the amount of the substance which has entered the cell can be made.

Remarks. The method is only applicable to slowly penetrating substances, and the fragility is directly dependent on pH , O_2 -tension and temperature, which must be accurately controlled. The permeability may be measured under physiological conditions and adsorption at the surface does not interfere with the results.

Changes in the Haemoglobin in the Cells. The classical example of the application of this method is the work of Hartridge & Roughton (1927) on the penetration of CO and O_2 into the cells, these gases causing changes in the light absorption of Hb (haemoglobin). In order to measure changes which occur in less than 1/50th of a second, the following method is used.

A solution of 1% NaCl, saturated with air at barometric pressure, is mixed in a special chamber (Hartridge & Roughton, 1923) with a suspension of oxygen-free sheep erythrocytes in 1% NaCl. The mixed suspension leaves the chamber and flows at a known rate through an observation tube, through which determinations of the percentage of HbO_2 were made at various distances along the tube. Knowing the rate of flow, the total gas-combining power of the Hb in the cells and the amount of O_2 in the suspension at the beginning of the experiment, the rate of combination of the O_2 with the haemoglobin may be determined, and, derivatively, the rate of penetration of the O_2 through the erythrocyte membrane. Thus the principle of the method is to convert time intervals into intervals of distance which can be measured with any desired accuracy.

Keilin & Mann (1941) have used the difference between the absorption spectra of acid and alkaline methaemoglobin to study permeability to anions.

Electrical Methods. These methods are based on (a) changes in the conductivity of the medium surrounding the cell due to leakage of electrolytes from the cell, (b) changes in the conductivity of a suspension of cells due to a change in their volume, (c) changes in the capacity or impedance of the cells due to a change in the membrane. (a) has been applied by Joel (1915) to suspensions of erythrocytes, (c) most notably by Fricke (1934), Fricke & Curtis (1935) and Cole & Curtis (1938). So far these methods have been of little value for any cells except for the large changes in permeability studied by Osterhout (1922) and Cole & Curtis (1938).

Arbacia punctulata. The unfertilised eggs of *Arbacia punctulata* are almost perfect spheres, about 70μ in diameter. When shrinking or swelling in hypertonic or hypotonic sea water, the eggs swell uniformly, simply changing their diameter. Volume changes in these eggs are very easily followed by a variety of physical methods.

If treated carefully the eggs as shed from the ovaries will withstand the necessary minimum of handling. Their chief disadvantage is that they are only available for a month or two of the year.

Methods. The simplest method is to observe changes in the diameter when the eggs are placed in solutions containing penetrating substances. For example, the permeability to water may be calculated from the rate of shrinkage in hypertonic, or swelling in hypotonic saline (McCutcheon & Lucké, 1928; Northrop, 1927 a, b; Jacobs, 1927). Then, if we wish to obtain the permeability to substances other than water (e.g. ethylene glycol), eggs, initially in sea water, are placed in sea water made hypertonic by addition of glycol; the eggs shrink as water moves out of the cell into the hypertonic solution. Ethylene glycol penetrates slowly into the cell and raises the tonicity of the cell interior, causing water to enter again, so that after some time the glycol is in equal concentrations inside and outside the cell, and the cell volume is restored to its original value. Under such conditions; then, the cell volume passes through a definite minimum. Jacobs & Stewart (1932) have shown that from the time at which this minimum is reached, the ratio of the rates of the penetration of water and glycol can be calculated, so that if we know the rate of penetration of water, we can calculate that of glycol.

Jacobs (1933) has given a more accurate treatment, taking into account changes in volume and cell surface area, which were

neglected in earlier work. This method of Jacobs is undoubtedly the best yet obtained. The permeability constants for water and for a second penetrating solute may be calculated by observing [a] the time at which the minimum volume is reached, [b] the value of the minimum volume, and consulting graphs drawn up by Jacobs. Hunter, using Jacobs' equation, has made calculations for the case where cells, added to an isotonic solution, swell from the beginning, so that the minimum volume method cannot be used.

The simplest way of measuring the changes in cell volume in these methods is to use the filar ocular micrometer, by which the diameter of the egg is measured directly. Alternatively, a diffraction method, due to Lucké *et al.* (1935), is available. A narrow parallel beam of monochromatic light is passed through a chamber containing a suspension of eggs, and the beam observed in a microscope. On either side of the main beam diffraction lines are found. The distance between these bands and the central beam is proportional to the diameter of the eggs. Consequently, changes in cell volume displace the position of the bands, and from these displacements the changes in cell volume may be calculated.

Plant Cells, etc. The cells of plants, such as *Elodea*, of yeasts and of bacteria have the great advantages of being readily available at most seasons of the year and of being fairly resistant to experimental treatment. Yeast and bacteria can often be obtained in suspensions of cells of fairly uniform size, so that it is possible to use large numbers of cells for each observation, as is the case with red cells. With plant cells, unless individual cells are used, it is usually only possible to make qualitative observations. Even when individual cells are studied, the shape of the cells imposes limitations on the use of physical methods, while chemical methods cannot be used because of the small size of the cells. Exceptions to this general rule are large cells, such as those of *Chara ceratophylla*, *Valonia* and *Nitella*, which are sufficiently large for accurate chemical measurements to be made on a single cell. These cells contain a large central vacuole, occupying most of the volume of the cell, and the protoplasm exists as a comparatively thin shell just inside the cellulose wall. Penetrating substances are usually estimated in the sap of the central vacuole, so that when such cells are discussed the term permeability frequently refers to passage of molecules not merely through the

external plasma membrane, but also through the protoplasm and the interior vacuolar membrane, which probably closely resembles the plasma membrane.

The plasmolysis method is that which was most widely used for plant cells during the last century, and it is on the basis of measurements using this method that Overton (1895, 1900) was able to make his important generalisation known as the lipid theory of permeability. If the osmotic pressure outside a plant cell is increased, water is withdrawn from the latter, and the protoplast, in consequence of the decrease in tension inside the cell, is detached from the cellulose wall (Fig. 4), i.e. the cell plasmolyses (Pringsheim, 1854). This process is easily observable under the microscope. If the osmotic pressure outside the cell has been raised by addition of a non-penetrating substance, the state of plasmolysis should be permanent; on the other hand, if the substance added was able to penetrate the cells, diffusion would occur until the original osmotic relations between the cell and its surroundings were re-established, so that the protoplast would return to its original position. The time required for this de-plasmolysis will be a rough measure of the rate of penetration of the substance added: if the latter penetrated very rapidly indeed the osmotic adjustments would not be brought about by appreciable movement of water out of the cell, so that the phenomenon of plasmolysis would not be observable.

Muscle and Nerve. Although their permeability should be of great interest, little work has been done on the cells of these tissues, and none of that published is quantitative. Fenn (1936) and Eggleton *et al.* (1937) have shown that Cl^- does not normally enter resting muscle cells. This observation provides the basis for a method for obtaining quantitative results on muscle. If a muscle is perfused with a slowly penetrating solute, the first thing that happens is that the solute passes through the capillary membrane into the interstitial spaces, fairly rapidly saturating the volume into which chloride ions penetrate. Penetration into the muscle fibres is much slower. Thus after perfusion for a moderate length of time, there will be sufficient solute to saturate the chloride space, and also some solute in the muscle fibres. If we estimate (a) the amount of chloride space, (b) the total solute in the muscle, then the excess of solute over that required to saturate the chloride space must have penetrated the fibres. By estimating the rate of

increase of this excess we can obtain permeability constants for the muscle cells. It is possible that a similar method may be applicable to nerve, but in the case of medullated nerve, interpretation of results may be ambiguous, owing to the difficulty of distinguishing between the effect of the medullating sheath and the plasma membrane.

GROUP II. COMPLEX MEMBRANES

Inanimate Membranes. C. M. Yonge has used chitin membranes, tying the membrane across the opening in a tube, so that the rate of diffusion into and out of the tube is measured. These membranes consist of a thin layer of lipoid superimposed on a porous chitin shell. They are unusually resistant to experimental damage. The shells of grasshopper eggs and of turtle eggs have also been the subject of interesting studies.

Frog Skin. Frog skin has been the subject of extensive studies, many of which are of little significance, since they were made on moribund or partly moribund tissue. It is probable that if the natural circulatory system cannot be maintained, the skin should for most purposes be perfused with a fluid of composition and colloid osmotic pressure which at least approximately correspond to those of frog blood. Soaking in Ringer's, for example, results in dilatation of the capillaries, to a diameter which may be twice or more than twice that of the normal. The permeability of such dilated capillaries is quite different from those of normal tissue. The melanophores are often contracted to a quite abnormal degree, and no doubt there are many other changes, which are less obvious, but equally important to the student of normal tissue. Even when properly treated, the results obtained on frog skin may well prove difficult to interpret, since the tissue consists of many layers of cells of diverse type and function.

The methods used up to the present usually involve clamping a section of frog skin between two tubes and then measuring either the potential difference across the skin, or the movement of substances from one tube to another.

Capillaries. Microscopic examination in the hands of such pioneers as Lewis and Krogh has provided the qualitative information which was necessary before a quantitative study could be made. Drinker (1927) has developed a semi-quantitative method for observing the web of the frog's foot. The tissue is

perfused through the femoral artery and a microscope is focused on the surface of the web. The rate of swelling of the web is proportional to the rate at which fluid moves out of the capillary, i.e. it is more or less proportional to the permeability of the capillary wall. As the web swells, its upper surface is raised, and the microscope must be adjusted to bring the web surface into focus. The time variation of the microscope focus gives a rough measure of the permeability of the capillaries.

Lewis & Grant (1925) and Krogh *et al.* (1932) and others have used a quantitative method based on the measurement of volume changes, which gives directly the excess of the fluid passing through the capillary wall in the arterial end of the capillaries over that reabsorbed in the venous end of the capillaries. This is an excellent method, especially as it may be applied to human subjects.* Danielli (1940) has simply weighed a tissue at intervals during perfusion; the capillary permeability may be calculated from the change in weight with time. This method has the advantage of technical simplicity and speed, but is not applicable to all subjects, and the results must be interpreted with caution.

The best method so far devised for accurate quantitative measurements of capillary permeability is that of Landis (1927). A single capillary is observed. At a suitable moment the capillary is clamped, and attention focused on a red cell in the capillary proximal to the blockage. As fluid filters through the capillary wall under the arterial pressure, the red cell moves up towards the blockage. From the rate of movement of the red cells and the dimensions of the capillary, the rate of filtration of fluid through the capillary can be calculated. The hydrostatic pressure in the capillary is determined by micro-cannulation, and with this information the permeability of the capillary can be obtained in absolute units. It should be pointed out that in these measurements the permeability of the capillary membrane to all the diffusible constituents of blood plasma is being measured, as opposed to the permeability of a membrane to a single species of molecule.

Kidney, etc. The kidney will be discussed in a separate chapter (xx). Other complex membranes, such as those of the intestine, etc., may be studied by isolating a loop of tissue, whilst maintaining the normal circulation. A solution containing the substance whose permeability is to be studied is placed in the

* See Dale & Richards (1918) with regard to the technique for isolated limbs.

loop, and absorption measured by analysing the material remaining in the loop after various times.

This method has been criticised by Cori (1925), on the grounds that isolation of a loop of intestine can upset its normal metabolism and so give illusory results; this author has worked out an extremely sound and reliable method of studying the absorption from the intestine in the intact animal by dividing a large number of rats into groups and taking the mean absorption from the intestines of each group, determined chemically after killing the animals, as a point on an absorption curve. Thus if fifty rats, divided into five groups of ten, are fed the same amount of glucose, after, say, 30 min. a group of ten rats is killed and the amount of glucose remaining in the intestines is determined. After a further period of time another group is killed, and so on. The results obtained in this way are remarkably consistent and it is unfortunate that this method is not more generally adopted.

In connection with such tissues it is important to realise that an apparent dependence of movement of a substance on oxygen pressure or some other feature of metabolism does not necessarily mean that active processes are involved, transporting the substance across the membrane by some process other than simple diffusion. It may simply mean that the structure of the membranes concerned is itself intimately linked with metabolism, so that metabolic factors may cause a simple diffusion process to simulate an active secretory process.

Use of Radio-Active Isotopes. Radio-active potassium, phosphorus, etc. should prove to be immensely useful in permeability studies. The chief restriction of their use is that at present small amounts of material only are available, but the amount of this type of work should increase greatly as more cyclotrons are installed. The method promises to be the most accurate and rapid available, *vide e.g.* Hahn *et al.* (1939), Brooks (1938, 1939).

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CHAPTER III

EQUILIBRIUM CONDITIONS OF CELLS

By H. DAVSON

PERMEABILITY studies are essentially studies of the adjustment of the cell to changes from its normal equilibrium conditions; thus, if we are studying a cell in its normal environment, and to this environment we add a new substance, or more of one of its constituents than normal, the equilibrium has been temporarily upset and the new position is re-established by the migration of molecules across the membrane. It is therefore necessary to devote some space to a consideration of these equilibrium conditions, the more so in the case of the erythrocyte, which is extremely sensitive to changes of temperature, pH , O_2 -tension and salt content of its surrounding medium, etc.; neglect of changes in equilibrium conditions may lead to most erroneous conclusions relating to permeability.

The discussion of equilibrium conditions is best begun by a few elementary considerations of the meaning of osmotic pressure.

Suppose that into a large volume of an aqueous solution of some substance, say sugar, B , is introduced a small volume, A , of a more concentrated solution of the same substance; after the lapse of a short time it is a matter of common experience that the sugar is no longer concentrated at A , but has become equally distributed all over B ; this mixing being achieved by the migration of the sugar molecules from A into B and also, and this is not always clearly recognised, by the migration of water molecules into A from B . If now A is enclosed with a membrane which is impermeable to sugar molecules, but permeable to water, it will be found that mixing will occur in these circumstances too, but only by the second mechanism discussed above, viz. by the migration of water molecules into A . If, however, A cannot increase in volume, then, of course, mixing will not occur, but the tendency of the water to mix with the sugar in A will be manifested by a pressure which may be measured by insertion of a manometer into A , and this pressure is known as the osmotic pressure difference of the sugar solution (Fig. 3). More exactly defined, it

is the pressure which must be exerted on the cell *A* to prevent water entering it from *B*. Given the concentrations of sugar in *A* and *B* and the temperature, the osmotic pressure difference may be calculated from the formula

$$P = RT (C_A - C_B), \quad \dots\dots(1)$$

where *P* = osmotic pressure difference,

R = gas constant,

C = molar concentration.*

The formula states that the osmotic pressure difference is determined by the difference between the number of molecules of solute in *A* and those in *B* per unit amount of solvent, and hence that the osmotic pressure difference becomes zero when the two concentrations become equal. It is to be noted that nothing is said in the formula about the nature of the molecules, so that if *A* contains glucose in a concentration of 1 mol per litre and *B* contains sucrose in the same concentration, and the membrane is impermeable to both these molecules, then there will be no difference of osmotic pressure between *A* and *B*.

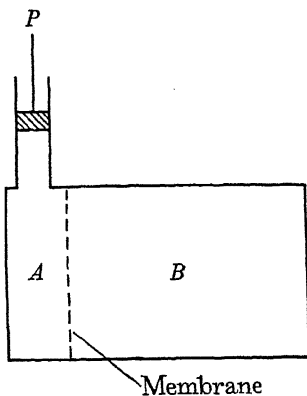


FIG. 3. Diagram to illustrate the behaviour of a semi-permeable membrane (see text).

If the membrane around *A* is elastic, mixing of water from *B* with the solution in *A* will be achieved by the penetration of water; in consequence the volume of *A* will increase and will continue to do so until the elastic tension in the membrane which opposes further expansion is just balanced by the difference in osmotic pressure.

This difference of osmotic pressure will, of course, be smaller than the original difference, since the solution in *A* has been diluted by the passage of water from *B*; if the membrane were

* For simplicity we have dealt with concentrations, not activities, of the components of a system. Strictly speaking, activities should be used, not concentrations, in the following discussion.

deficient in tensile strength one can imagine the possibility that it would burst before the equilibrium condition defined above were achieved.

It is now evident that *A* may adapt itself to an osmotic pressure difference between its own fluid and that in *B* in two ways. First, by structural resistance to an increase in volume which opposes the tendency for water to migrate into *A*, and secondly, by a reduction of the osmotic pressure difference, which is achieved by allowing water to penetrate, thereby diluting the solution within it.

In dealing with the osmotic properties of solutions of electrolytes two points must be borne in mind: first, that electrolytes dissociate in aqueous solution into ions, and that each ion in dilute solution is osmotically equivalent to an undissociated molecule; hence 1 *M* NaCl has roughly the same osmotic pressure as 2 *M* glucose, and 1 *M* K_2SO_4 has roughly the same osmotic pressure as 3 *M* glucose. Secondly, it is only necessary for a membrane to be impermeable to a single species of ion, i.e. a cation or an anion, for it to be impermeable to the salt of which this ion is a part. Thus if *A* in Fig. 3 contains NaCl solution and *B* KCl, and the membrane is impermeable to positive ions, i.e. Na^+ and K^+ , no diffusion of salt from one side of the membrane to the other can occur; chloride ions can of course pass across the membrane, but the passage of one from *A* to *B* must be associated, on the average, with the passage of one from *B* to *A* to maintain electrical neutrality, so that there is no passage of salt across the membrane. This point is important, as it is found that the impermeability of many biological membranes to salts is achieved by this form of specific ionic permeability.

Bearing these points in mind, two simple biological systems may now be approached. First, a typical plant cell bathed in a large volume of salt solution may be considered. This will consist (Fig. 4 (*a*)) of the cellulose wall, a rigid structure which is permeable to both salts and to water; the protoplast, which is lined with a non-rigid plasma membrane and consists of fluid or semi-fluid material; and finally, the central vacuole full of sap, consisting essentially of a salt solution. Since the plasma membrane of the protoplast may be considered as being salt impermeable, it is clear that we have a system similar to that in Fig. 3; the sap represents the contents of *A*; the protoplast, although complex,

may be represented as a single membrane, and the surrounding salt solution can be considered as the fluid *B*.

If the concentration of the surrounding salt solution is less than that in the sap, there will be a difference of osmotic pressure tending to drive water into the cell; this tendency will be resisted, however, by the structural rigidity of the cellulose wall and therefore very little, if any, increase in volume of the sap will be observed. If, on the other hand, the concentration of the surrounding fluid is increased above that in the sap, water will pass out of the cell, the volume of the sap will diminish and the protoplast will detach itself from the cellulose wall (Fig. 4 (*b*)). The space between the protoplast and the cellulose wall, formed thus,

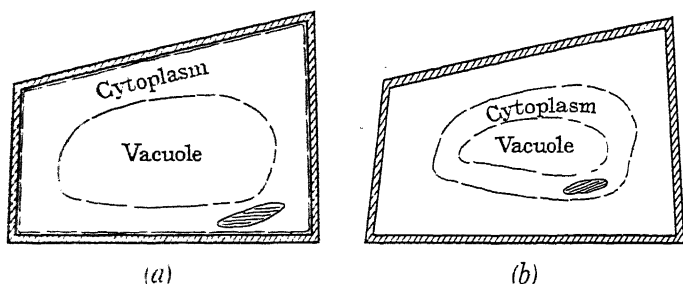


FIG. 4. Plant cell in (*a*) hypotonic saline, (*b*) hypertonic saline (plasmolysed).

is filled with fluid; the condition at equilibrium will be that the vacuole has lost sufficient water to bring its concentration up to that of the surrounding fluid. Thus in the former instance the adaptation consisted exclusively in the exertion of a structural resistance to an increase in volume, whilst in the latter case the adaptation was brought about by the migration of water from the sap to the surrounding fluid until the concentration of dissolved substances in the sap was made equal to that in the surrounding fluid. The phenomenon of the detachment of the protoplast accompanied by a shrinkage of the sap space is known as *plasmolysis*.

Suppose now the solution outside the cell is made hypertonic, i.e. its concentration greater than in the sap, by the addition of a substance which penetrates the cell slowly in comparison with the rate of migration of water. In this instance, the tendency for

osmotic pressures to equalise can be gratified in two ways, first, by the passage of water out of the cell, and secondly, by the passage of the added substance into the cell. The passage of water out will cause plasmolysis and a temporary equilibrium will be achieved with roughly equal osmolar concentrations on each side of the membrane. However, the added substance can penetrate the cell, and as it does so it increases the osmotic pressure inside the cell, thereby causing water to penetrate also, and this process will go on until the sap volume has increased to its original value. Consequently, under these conditions, addition of a penetrating substance to the surrounding medium causes first plasmolysis and then recovery, the latter being known as de-plasmolysis. If the rate of penetration of the added substance is sufficiently large in comparison with that of water, it can happen that equality of concentration on each side of the membrane is achieved almost exclusively by migration of the substance across the membrane, so that the passage of water in the opposite direction is negligible; in this event the addition will not cause plasmolysis.

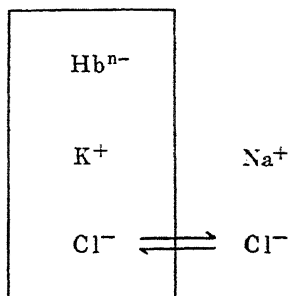


FIG. 5. Diagram illustrating equilibria in the red cell.

Passing now to the second biological system, let us consider the erythrocyte. For the moment its ionic contents and those of its surrounding fluid may be considered simply as in Fig. 5. The membrane is permeable to anions but not to cations, hence it may be said that the membrane is impermeable to salts, since no transfer of NaCl or KCl is possible. If the concentration of NaCl is reduced, a difference of osmotic pressure will be produced and water will tend to pass into the cell; since the erythrocyte can increase in volume, water will actually pass into it, and the position of equilibrium will be given by the condition that the osmotic forces are balanced by the elastic forces in the membrane. Now, as the elastic tension which the erythrocyte membrane can oppose to osmotic forces is to all intents and purposes zero, the position of equilibrium will be given only when $\Delta P \simeq 0$, i.e. when equality of osmotic pressure on each side of the membrane occurs, this equality being achieved by the passage of water into the cell,

which dilutes its contents. If the NaCl concentration is reduced to $0.10 M$, it is found that the cell can accommodate the water required for this neutralisation of osmotic pressure; if the NaCl concentration is reduced much below this value, say to $0.08 M$, it is found that the capacity of the cell is insufficient for the water necessary to reduce the concentration of KCl in the cell to $0.08 M$ and consequently the cell bursts, i.e. *haemolysis* takes place. Hence we see that the erythrocyte's only way of adapting itself to a reduced osmotic concentration of salts outside it is to swell until the concentrations on both sides of the membrane are equal; unlike the plant cell it can offer practically no structural resistance to the osmotic forces. In hypertonic solutions of NaCl the levelling out of the osmotic forces will of course be achieved by the shrinking of the cell.

We have already seen that the conditions for osmotic equilibrium across a membrane are independent of the nature of the molecules on either side so long as the membrane is impermeable to them; thus if the NaCl is replaced with $0.33 M$ glucose to which the erythrocyte is impermeable, there will be osmotic equilibrium and no passage of water will occur; if, however, the cell is placed in an isotonic solution of glycerol, which can penetrate the membrane, we shall have initially the same osmotic pressure on both sides of the membrane; but in this case the mixing tendency of glycerol with the cell contents can be gratified and glycerol will therefore pass into the cell. In so doing it raises the osmotic pressure of the cell contents, water passes in to reduce the osmotic pressure, and it will be clear from Fig. 6 that no stable osmotic equilibrium can be achieved until the cell bursts and allows the KCl to escape, since as fast as glycerol penetrates the cell its concentration is reduced by the simultaneous penetration of water. Hence placing the erythrocyte in a pure solution of a penetrating substance will cause *haemolysis*.

If the erythrocyte is placed in isotonic NaCl to which has been added glycerol in a concentration of say $0.1 M$, the cell will at first shrink, owing to the excess of osmotic material outside it. Then, as glycerol penetrates, it will regain its original volume—but in these circumstances it will not swell up and burst, since an equilibrium position, in which the osmotic pressures are equal and the glycerol is distributed in equal concentration on both sides of the membrane, can be achieved (Fig. 7), whilst in the case of

the cell suspended in a pure solution of isotonic glycerol without added salts this was not possible. The proportions of NaCl and penetrating substance in the solution surrounding the cell

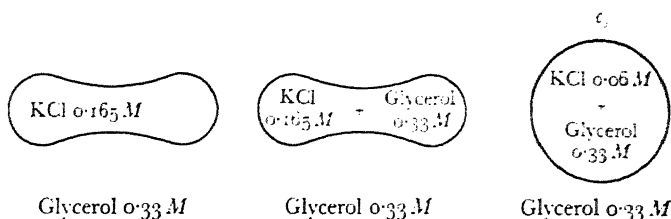


FIG. 6. The swelling of a red cell in isotonic glycerol. (a) The cell has been placed in 0.33 M glycerol. (b) Imagine that the glycerol has achieved osmotic equilibrium and that no water has yet entered the cell. The difference in concentration is equivalent to 0.165 M KCl. (c) Imagine that H_2O has migrated until the KCl concentration is 0.06 M; at this stage the cell is ready to burst and we note that there is still a difference in concentration 0.06 M, since more glycerol has penetrated with the water.

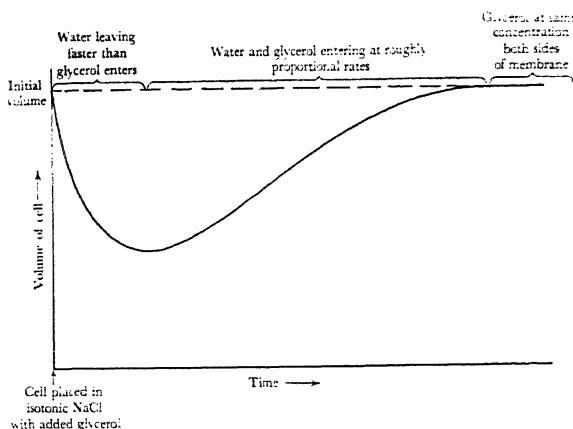


FIG. Shrinking of a red cell followed by swelling when glycerol is added to isotonic saline.

necessary to maintain the cell intact will be determined by the amount of water which the cell can take up. Thus if 0.10 M NaCl + 0.065 M glycerol is the suspension medium for the cell, the equilibrium position will be as in Fig. 7, i.e. the system will be stable, since the cell can in general take up enough water to

reduce the concentration of salts inside it to $0.10 M$. However, if the concentration of $NaCl$ were $0.08 M$ or less, the cell would be unable to take up the necessary water and so haemolysis would occur; the rate at which the haemolysis occurred would be proportional to the rate of penetration of the glycerol. The limiting concentration of $NaCl$ or other non-penetrating substance in the mixture below which haemolysis occurs will be given by the critical haemolytic concentration of the cell.

In this discussion of the osmotic properties of the erythrocyte attention has been confined to a single cell; in actuality, of course, a single cell is only rarely studied and in general many millions of cells are used for a single measurement. If a sample of erythrocytes is taken and placed in a $NaCl$ solution whose concentration is now gradually reduced, it will be found that at a certain concentration of the $NaCl$ some of the cells will haemolyse whilst the remaining ones will still be intact; as the concentration of $NaCl$ is further reduced more cells will haemolyse until eventually all will have done so. If the degree of haemolysis is plotted against the concentration of $NaCl$ we obtain a "Percentage Haemolysis Curve" (Fig. 2). It has already been seen that the rate at which a cell swells when a substance is penetrating it could possibly be used as an index to the rate of penetration of the substance; now it is not easy to measure the swelling of a single erythrocyte, but it is easy to measure the degree of haemolysis, i.e. the percentage of cells which have burst, of a cell suspension containing many cells. Consequently, a suspension of cells is looked upon as a single cell in many permeability studies, so that if, under given conditions of the penetration of water, for example, a certain degree of haemolysis is observed to occur in time t , the latter is looked upon mathematically as the time required for a certain amount of water to enter this single hypothetical cell. Thus, if erythrocytes are placed in a strongly hypotonic $NaCl$ solution, e.g. $0.04 M NaCl$, and the degree of haemolysis is measured at definite intervals, a rate of haemolysis curve may be plotted as in Fig. 8, and from this curve, on the basis of certain assumptions which need not be entered into, the rate of penetration of water into the erythrocyte may be measured. It should be recognised clearly that the rate at which a certain degree of haemolysis is attained depends not only on the rate of penetration of the water but also on the amount of water which the individual erythrocytes

can accommodate without bursting, so that if a comparison is to be made between the rates of penetration of water under two different sets of conditions, say in the presence or absence of a narcotic, one must make absolutely sure that there is no difference in this quantity of water which the erythrocyte can take up without bursting, i.e. the critical haemolytic volume, under the two conditions, or if there is, that allowance is made for it.

Factors determining the nature of the percentage haemolysis curve (Fig. 2) of the erythrocyte will be discussed more fully in

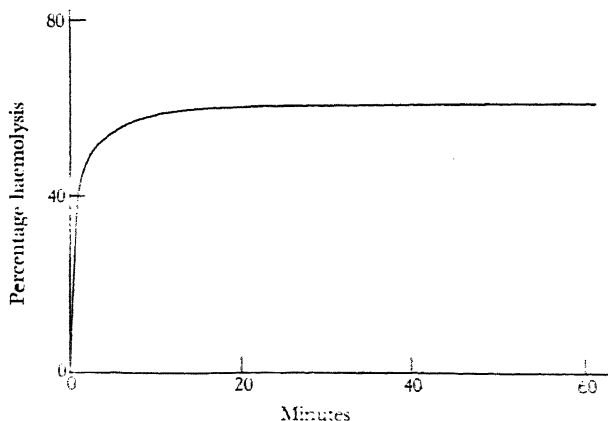


FIG. 8. A typical "rate of haemolysis" curve for red cells in $0.09\text{ }M$ NaCl (after Jacobs & Parpart, 1932).

the chapter on haemolysis, but there is one point which should be brought out here, since it forms the basis of a very neat method of measuring permeability. Other things being equal, it will be obvious that the degree to which the concentration of salt surrounding the erythrocyte may be reduced, before the latter haemolyses, will be determined by the concentration of salts in it; thus, if an erythrocyte which contains $0.165\text{ }M$ salts haemolyses in $0.09\text{ }M$ NaCl, an erythrocyte with exactly the same properties, but which contains $0.200\text{ }M$ salts, will haemolyse in $0.109\text{ }M$ NaCl; consequently, if erythrocytes are suspended in a saline solution containing a penetrating substance and after various periods of time samples are removed and the concentrations of NaCl at which haemolysis occurs determined, it will be found that these

concentrations progressively increase as more and more of the penetrating substance enters the cells. The rate at which these changes occur will be a fair measure of the rate of penetration of the substance studied.

There is an important condition regarding the distribution of ions across a membrane defined by the Gibbs-Donnan equilibrium. Let us consider the system shown in Fig. 9; The membrane, M , separates two aqueous solutions, (i) containing NaCl and the sodium salt of a protein, Na^+P^- , and (ii) containing only NaCl . The membrane is permeable to Na^+ and Cl^- , but not to the

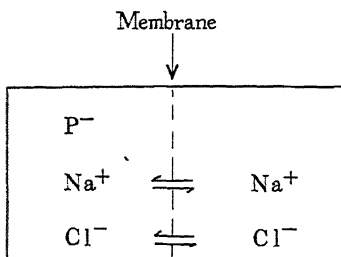


FIG. 9. Illustration of the Gibbs-Donnan equilibrium. The membrane, M , is permeable to all ions except P^- .

protein ions, P^- . The problem is to find how the diffusible ions, Na^+ and Cl^- , will be distributed at equilibrium. Gibbs, and later Donnan, have shown that the products of the concentrations of the diffusible ions on each side of the membrane will be equal. If, for convenience, it is assumed that the volumes of solutions (i) and (ii) are equal to unity and that the experimental conditions are such that they remain constant, and if, further, we designate the numbers of ions in the two volumes by Na_1 , Cl_1 , P , Na_2 , Cl_2 , the condition of equilibrium will be given by the equation:

$$\text{Na}_1 \times \text{Cl}_1 = \text{Na}_2 \times \text{Cl}_2. \quad \text{.....(2)}$$

Since $\text{Na}_1 > \text{Cl}_1$ and $\text{Na}_2 = \text{Cl}_2$, it follows that

$$\text{Na}_1 + \text{Cl}_1 > \text{Na}_2 + \text{Cl}_2,$$

$$\text{or} \quad \text{Na}_1 + \text{Cl}_1 = \text{Na}_2 + \text{Cl}_2 + n, \quad \text{.....(3)}$$

where n is equal to the number of excess ions on side (i) of the membrane.

This equation shows us that there will be a difference of osmotic pressure between the two solutions in virtue of the excess of ions in solution (i) over those in solution (ii). Furthermore, on the side (i) there will be protein ions which will also exert an osmotic pressure. Hence the total number of osmotically active ions on side (i) will be $\text{Na}_2 + \text{Cl}_2 + n + P$.

On side (ii) there will be only $\text{Na}_2 + \text{Cl}_2$.

Hence the total difference of osmotic pressure between (i) and (ii) will be given by $(n + P) \times R \times T$; the direction being such as to drive water from (ii) to (i). This difference of osmotic pressure is spoken of as the "Colloid Osmotic Pressure" of the protein solution. Since there must always be a difference of osmotic pressure between two solutions typified by (i) and (ii) in Fig. 9, it is clear that an equilibrium can only be established either by the passage of all solution (ii) into solution (i) or by the enclosure of solution (i) in a chamber capable of exerting a structural or hydrostatic resistance to the penetration of fluid. Thus if a protein NaCl solution is enclosed in a collodion sac, permeable to NaCl but not to protein molecules, with a tube in the top and submerged in a solution of NaCl, it will be found that owing to the difference of osmotic pressure water will pass into the sac until the fluid has risen in the tube to a height which creates a hydrostatic force large enough to balance the osmotic force.

The state of affairs envisaged in Fig. 9 is found in plasma and certain body fluids, where a protein-containing fluid, the plasma, is separated from a solution containing less protein by a membrane, the capillary endothelium, which is permeable to salts but relatively impermeable to the plasma proteins. In these circumstances, the difference of osmotic pressure which tends to drive water from the tissue fluid into the plasma is counterbalanced by the hydrostatic pressure in the capillaries.

Returning to equation (i), we find

$$\text{Na}_1/\text{Na}_2 = \text{Cl}_2/\text{Cl}_1 = r,$$

where r is greater than 1.

Hence, with a system defined by Fig. 9, it will be found that not only is the osmotic pressure of (i) greater than that of (ii) but that there will be in solution (i) an excess of Na above that in solution (ii) and a deficiency of Cl below that in the same solution. Van Slyke (1926) has calculated that the value of the

Gibbs-Donnan ratio, r , in the case of blood plasma and oedema fluid should be $1.0 \pm$: a value which agreed fairly well with the experimental values of Loeb *et al.* (1922); similar relationships appear to hold in regard to the aqueous humour (*vide e.g.* Davson, 1939), ascitic fluid (Muntwyler *et al.* 1931) and possibly synovial fluid (Ropes *et al.* 1939).

Passing now to the erythrocyte, a few points which bear on permeability studies will be entered into: for an exhaustive treatment of the equilibria of the erythrocyte the reader is referred to E.J. Warburg (1922), Van Slyke (1926) and Henderson (1928). Fig. 10 represents schematically the state of affairs in the inside and outside of the erythrocyte; the protein content of the plasma is neglected and the small quantities of other cations than Na in the plasma are also ignored. As we have seen, the cell membrane is permeable to anions and impermeable to cations. The concentration of haemoglobin inside the cells is about 30% of their weight, a very considerable amount in comparison with the protein contents of other cells; the presence of this protein,

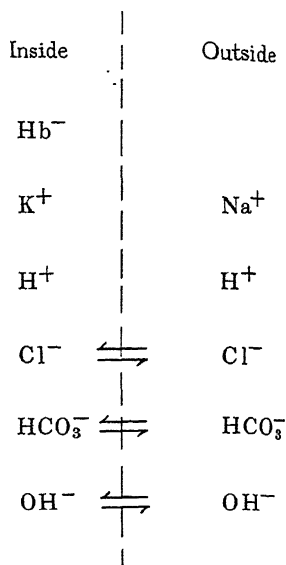


FIG. 10. Diagram of equilibria between the red cell and saline.

which at physiological reaction dissociates as an acid, gives a system defined by the Gibbs-Donnan equilibrium, and may be expressed in relation to the diffusible anions thus:

$$\text{Cl}_i/\text{Cl}_o = (\text{HCO}_3)_i/(\text{HCO}_3)_o = \text{OH}_i/\text{OH}_o = r, \dots\dots(4)$$

where the suffixes i and o refer to the inside and outside of the cells respectively. The ratio, r , is under normal conditions about 0.8, so that if the chloride concentration in the plasma is 0.10 M the concentration in the cells will be 0.08 M . The uneven distribution of ions is, of course, dependent on the degree of ionisation of the haemoglobin; the more potassium haemoglobin in the cells in relation to KCl and KHCO_3 , the smaller will be the value

of r , and consequently the lower the concentration of chloride in the cells. A failure to realise this point among other things led Mond (1927) to his erroneous claims regarding the reversal of ionic permeability at alkaline reaction, as will be seen later.

Now the most important consideration from the practical point of view of permeability is the following. The ionised haemoglobin in the cells is in the form of a polyvalent anion at physiological reaction, K_nHb , so that one mole, say, of the K salt of haemoglobin, on dissociation into ions, will give $nK^+ + 1 Hb^-$. If the same quantity of K were bound as KCl or $KHCO_3$, we should have $nK^+ + nCl^-$ or $nK^+ + nHCO_3^-$, i.e. the total number of ions in the latter case would be larger; hence any influence of the cell environment which causes K_nHb to be formed in place of KCl and $KHCO_3$ will decrease the total number of ions in the cell and thereby reduce the osmotic pressure of its contents, causing the cell to shrink. Increased alkalinity, oxygen tension, and temperature will all tend to act in this way since they increase the amount of K_nHb in the cells. Jacobs & Parpart (1931) have derived the following simple equation to express the dependence of the water content of the cell, i.e. the degree of swelling, on the base bound by the haemoglobin:

$$\frac{W_1}{W_2} = \frac{2R+1-F_1}{2R+1-F_2}, \quad \dots\dots 5$$

where W_1 and W_2 are the water contents of the cell under two conditions, e.g. of O_2 -tension or temperature; R is the ratio of base in the cells to haemoglobin; F_1 and F_2 are the amounts of base bound by unit volume of haemoglobin under the conditions corresponding to W_1 and W_2 .

The formula has been submitted to experimental test by these authors in the case of the ox erythrocyte and a remarkably good agreement with theory was found: however, an increasing amount of evidence is accumulating which tends to show that the effect of temperature, e.g., on the swelling of the erythrocyte is not to be completely explained on the basis of an increased salt formation of haemoglobin at higher temperatures, as the simple treatment, exemplified by the above equation, would demand. Thus Jacobs *et al.* (1936) have shown that the shrinking of erythrocytes due to raising the temperature from 20° to 40° is in many species irreversible, so that the cells do not return to their original volume

on reducing the temperature to 20° again. Davson (1937) has shown that these changes are too rapid to be accounted for by an escape of cations from the cell at the higher temperature. Again, Ponder (1934) has argued repeatedly that the osmotic behaviour of the rabbit erythrocyte is not to be characterised by the simple theoretical treatment of Jacobs & Parpart, and this author postulates a variable escape of potassium from the erythrocyte during swelling, which would account for some of the anomalies in its osmotic behaviour (Ponder & Robinson, 1934); here again, however, chemical determinations by Davson (1936) have failed to reveal losses of potassium of the order expected.

Whatever the fundamental cause may be, it has to be remembered by all students of the permeability of the erythrocyte that changes in the pH , O_2 -tension and temperature may have considerable effects on the degree of swelling of the erythrocyte. It has been seen in Chapter II that the most commonly used techniques for studying permeability in the erythrocyte are based on rates of osmotic haemolysis, in general the time required for a given degree of haemolysis to occur being used as an index of this rate. The time required for this degree of haemolysis to occur will obviously depend, other things being equal, on the amount of water already in the cell, and since we have already seen that this quantity is dependent on temperature, pH and O_2 -tension, is clear that these quantities must be rigidly controlled when using the haemolysis technique for measuring permeability.

The "Hamburger Shift". This phenomenon, although not very important from a permeability point of view, may be briefly described, since adequate explanations are not always to be found in the physiology texts. The "Hamburger Shift" consists in the migration of chloride into the cells when the plasma is made acid with CO_2 or some other acid, and the migration of HCO_3^- from the cells to the plasma at the same time. Loss of CO_2 or addition of alkali have the reverse effect. The presence of excess CO_2 in the plasma causes diffusion of this molecule into the cells; here the CO_2 unites with H_2O in the presence of carbonic anhydrase (Meldrum & Roughton, 1933) to form H_2CO_3 ; the latter unites with K_nHb to give $KHCO_3$, which, being a dissociated salt, increases the HCO_3^- concentration in the cells. To establish equilibrium, some of the extra HCO_3^- must diffuse into the plasma, and as the membrane is permeable to anions only, this

must be achieved by the simultaneous movement of Cl^- from plasma to cells. The reverse chain of processes will occur when CO_2 is lost from the plasma.

It may be argued that whilst the passage of HCO_3^- from cells to plasma satisfies the tendency for these ions to distribute themselves in accordance with their equilibrium position, the passage of Cl^- in the opposite direction will actually represent movement away from equilibrium. In actuality this is not so; the equilibrium position is given by

$$\frac{(\text{HCO}_3)_i}{(\text{HCO}_3)_o} = \frac{\text{Cl}_i}{\text{Cl}_o} = r.$$

Increasing the acidity will cause an increase in the value of r , the Gibbs-Donnan ratio, hence Cl_i must increase at the expense of Cl_o , i.e. Cl^- must penetrate the cells.

TABLE I. THE DISTRIBUTION OF VARIOUS IONS BETWEEN THE INSIDE AND OUTSIDE OF A RED CELL AFTER DILUTION OF THE PLASMA WITH ISOTONIC NON-ELECTROLYTE (JACOBS & PARPART, 1933)

	Dilution = 100-fold	Dilution = 1000-fold
V_c	0.972	0.853
$(\text{Cl})_o$	6.97×10^{-5}	3.68×10^{-5}
$(\text{Cl})_i$	7.51×10^{-2}	5.07×10^{-2}
$\text{Cl}_i / \text{Cl}_o$	1.08×10^3	1.37×10^3
H_i	6.47×10^{-8}	2.68×10^{-8}
H_o	6.97×10^{-5}	3.68×10^{-5}
H_o / H_i	1.08×10^3	1.37×10^3

V_c is the relative volume of the cell, the initial volume being unity. The suffixes i and o refer to the inside and outside of the cell respectively.

There is a special case obtaining when the cell is suspended in a large volume of a non-electrolyte solution; under these conditions there will be a large concentration difference of Cl^- and HCO_3^- in the direction cells \rightarrow suspension medium; however, these ions can only diffuse out of the cell if other ions of the same charge diffuse in at the same time: in a non-electrolyte solution the only ion available is OH^- , so that a certain amount of this ion will diffuse into the cell and thus make the cells alkaline and the suspension medium acid (Netter, 1928). This acidity of the non-electrolyte medium on addition of erythrocytes to it had been observed by Coulter (1925), but was interpreted differently. Jacobs & Parpart (1933) have treated the matter theoretically on the basis of Netter's explanation, and in Table I their calculated

values for the concentrations of some of the constituents of the erythrocyte on each side of the membrane are given for dilutions of blood in the non-electrolyte medium of 1 : 100 and 1 : 1000. If their calculations (which are only approximate) are of the right order, there should be a pH difference between the inside and outside of the cell of about 3 units when the blood is diluted one in a thousand. Since in these circumstances the cell contents become alkaline, it follows from what went before that the cells will shrink on being transferred to an isotonic non-electrolyte solution, and consequently the amount of a penetrating non-electrolyte which has to enter the cells to cause haemolysis will be materially increased, so that great care must be exercised in interpreting rates of haemolysis in terms of penetration.

It has been seen that the reduction of the number of ions inside the erythrocyte produced by the formation of K_nHb at the expense of KCl and $KHCO_3$ causes the cell to shrink. A shrinking of a similar nature may also be caused by replacing the monovalent anions in the cell by extraneous polyvalent anions. Thus if cells are suspended in isotonic Na_2SO_4 , the SO_4^{--} ions will diffuse into the erythrocyte and two monovalent anions must diffuse from the erythrocyte in exchange for each SO_4^{--} ion which enters. The net effect of this exchange will be to reduce the total number of ions in the erythrocyte, and the latter will shrink so that osmotic equality on both sides of the membrane is attained: the shrinkage obtained in this way could be used as a qualitative guide to the permeability of the erythrocyte to certain polyvalent anions (Parpart *et al.* 1937). If the extraneous anion penetrates with difficulty, it will be found that the Cl^- and HCO_3^- in the cell diffuse out in exchange for OH^- ; hence the suspension medium becomes acid. Thus suspension of cells in isotonic sulphate solution gives a reaction of pH 7.0, whereas in isotonic chloride solution the pH is 7.6; as SO_4^{--} penetrates the cell the reaction becomes more alkaline (Davson, Wilbrandt, 1939).

In the discussion of the Gibbs-Donnan equilibrium pertaining between plasma and interstitial body fluids, it was pointed out that a difference of osmotic pressure between the two fluids must exist; in the case of the erythrocyte, however, it is clear that no such permanent difference of osmotic pressure between its contents and its surroundings can be present, otherwise the cell would be unstable, since its membrane cannot resist any appreciable

osmotic pressure difference; yet the colloid osmotic pressure of the cell contents must be very much greater than that of plasma. The absence of such a difference of osmotic pressure is due entirely to the indiffusibility of the cations, which allows of an unequal distribution of anions as demanded by the Gibbs-Donnan equilibrium and at the same time an equal osmotic concentration on both sides of the membrane. If, due to some environmental influence, the erythrocyte were to become permeable to sodium and potassium, then of course it would swell up and haemolyse (*vide* e.g. Davson & Danielli, 1938; Davson & Ponder, 1940).

Equilibrium Conditions in Muscle. As a result of the careful and painstaking work of Fenn and his collaborators, the previously scattered information regarding the equilibria in muscle has been integrated into a fairly well-defined picture of the ionic relationships between the cell and its environment. The muscle contains an intercellular space which, on the basis of anatomical studies, amounts to 15% of its weight (Hermann, 1888 : this space is filled with interstitial fluid which will be expected to have the same composition as an ultra-filtrate of plasma. Chemical determinations of the composition of muscle indicate that all the chloride present is

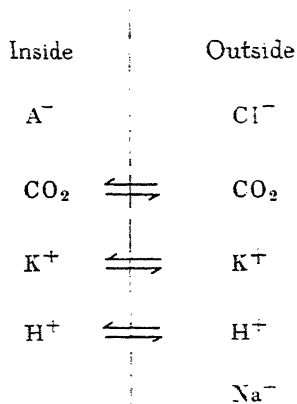


FIG. 11. Equilibria between the muscle cell and saline.

in this interstitial fluid, the rest of the muscle being free from this ion. If this is the case, the amount of fluid which must be present in the muscle to contain the chloride found represents on the average 14.7% of the muscle weight (Fenn *et al.* 1934); this weight of fluid is called the "chloride space". Practically all the potassium in muscle, on the other hand, has been found to be concentrated in the muscle cells. The ionic conditions in muscle and interstitial fluid may consequently be represented as in Fig. 11. A^- represents the sum of the anions, consisting of phosphates, lactate, etc. in the muscle cell. For such a system to be stable, i.e. in order that the concentrations should

not level themselves out by diffusion, the membrane must be impermeable to some of the ions present; the general consensus of opinion would indicate that the membrane, at rest at least, is impermeable to all the anions present, and also to sodium. We thus have a condition defined by the Donnan equilibrium, so that we may write with regard to the diffusible ions:

$$\frac{K_i}{K_o} = \frac{H_i}{H_o}.$$

Since the concentration of potassium in the muscle cell is approximately twenty-five times that in the interstitial fluid, it is clear that a difference in pH between the inside and outside of the muscle cell must exist: Fenn & Maurer (1935) have allocated a value of pH 6.9 to the interior of the muscle cell, indicating a difference of pH of not more than 0.5 unit, i.e. much less than is demanded by the ratio of the potassium concentrations. Similarly, it was found that the potential difference between the inside and outside of the muscle cell, the resting or injury potential, which should be given by the formula $RT/F \ln K_i/K_o$, is about half the expected value, but this could be explained on the grounds that short circuits occur during measurement. As Fenn (1936) states, the present simple picture of the ionic equilibria between the muscle cell and its environment is not adequate to cover all the known facts, but as a working hypothesis it must be retained until some other and more adequate theory is presented. The normal impermeability of the muscle cell membrane to Na^+ seems to be well established: the evidence with regard to phosphates, Cl^- and other anions is conflicting; the permeability of the membrane to K^+ is beyond all reasonable doubt.

Under the conditions outlined above, osmotic equality between cell and interstitial fluid should exist, in spite of the colloid osmotic pressure of the cell contents (according to Duff, 1932, this is equal to about 45 mm. Hg), since the membrane is anion impermeable. Any disturbance of the normal impermeability of the muscle-cell membrane to anions or to sodium would, however, bring about an osmotic pressure difference and the cells would swell; this accounts for the common observation that injurious influences generally cause a swelling of the muscle. In this discussion, the interstitial or chloride space has been treated as a single entity; functionally it may be divided into a capillary

space which will be filled with blood (ca. 2–10% of the muscle weight, Danielli, 1941; Danielli & Davson, 1941) and a true intercellular space. In permeability studies it is often essential to realise this distinction since, in a perfused muscle, a substance entering the muscle cell from the blood must penetrate first the capillary endothelium and then, after diffusion through the interstitial space, the muscle-cell membrane.

TABLE II. ELECTROLYTE ANALYSES OF SQUID NERVES EXPRESSED IN MILLI-EQUIVALENTS PER GRAM OF WATER IN AXOPLASM, BLOOD AND SEA WATER (BEAR & SCHMITT, 1939)

Substance	Axoplasm m. eq. per gram	Blood m. eq. per gram	Sea water m. eq. per gram
Total base	0.62	0.67	0.54
K	0.31	0.017	0.012
Cl	0.13	0.53	0.51
Total base – K (chiefly Na)	0.31	0.65	0.53
Total base – Cl (anion deficit)	0.49	0.14	0.03
Total base – Cl (total ions analysed)	0.75	1.20	1.05

This discussion of ionic and osmotic equilibria has centred itself mainly on the erythrocyte for the reasons that the information regarding it is more complete than with any other cell, that the permeability of this cell has been more consistently studied than any other, that the high concentration of haemoglobin in the erythrocyte makes the study of its equilibria of great importance for permeability phenomena, and finally for the reason that the equilibria in this cell are not appreciably complicated by metabolic activity (Davson & Danielli, 1938). So far as other cells are concerned, it may be stated that, as far as we know, the conditions in nerve are essentially the same as those in muscle, having regard for the differences in structure of the tissues (Macdonald, 1902, 1905; Cowan, 1934). Recent analyses of Bear & Schmitt (1939) on the single axons of squid nerve fibres (Table II) emphasise, however, that the nerve cell does contain chloride and, furthermore, that there is a deficiency of anions in the nerve cell, i.e. the total concentration of anions required for electrical neutrality of the solution as a whole is not made up by the sum of the individual concentrations of anions so far isolated and determined. These authors suggest that the unidentified anion will be found to have an atomic weight of about 100. The egg of *Arbacia*, a sea-urchin, is probably only slightly permeable, if not impermeable, to

anions (Jacobs & Stewart, 1936), and this also seems to be true of plant cells, *vide e.g.* Jacobs (1922); in fact the rapid permeability to anions which characterises the erythrocyte appears to be a peculiarity of this cell* and represents an adaptation to the special functions of the erythrocyte as an oxygen carrier and the principal component in the regulation of the pH of the blood. Harvey (1932) has made a valuable collection of information regarding the chemical composition of *Arbacia* eggs, and similarly Krogh (1939), on a much larger scale, has collated a great deal of information regarding osmotic regulation in aquatic animals, to which the interested reader may be referred.

Note added in proof. Since this chapter was written, a valuable paper by Boyle & Conway (*J. Physiol.* 1941, 100, 1) has appeared describing experiments which demand a complete re-orientation of commonly held views on muscle permeability and potentials. Apparently the muscle-cell is permeable to chloride and the very low concentration of this ion in the cell under normal conditions is due to the presence of non-diffusible organic anions. This paper should certainly be consulted by all interested in muscle equilibria.

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* One of us (J.F.D.) believes it to be equally probable that many cells are permeable to anions to at least the same degree as to cations.

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CHAPTER IV

SOME EQUATIONS USED IN PERMEABILITY STUDIES

By H. DAVSON

IN this chapter we wish to discuss as simply as possible the mathematical representation of the permeability process, so that intending workers in the field of permeability may have an adequate notion of the real meaning of the permeability constant and of the justification, or otherwise, of some of the short cuts by which measures of permeability are obtained without the use of the permeability constant as defined below.

The migration of a substance in solution into or out of a cell is a problem of diffusion, so that Fick's Law, which says that the rate of diffusion across a given cross-section is proportional to the concentration gradient at that point, should apply. The application of this law becomes comparatively simple when it is assumed that the concentration gradient across the membrane is the only gradient determining the rate of penetration; this will be very nearly true if the membrane slows the normal rate of diffusion in an aqueous medium to any great extent, especially if stirring is carried out in the suspension medium. Fick's Law may then be stated thus:

Rate of penetration

$$= kA \frac{\text{Concentration difference between cell and medium}}{\text{Thickness of membrane}}.$$

If the thickness of the membrane is considered constant, it may be incorporated in the diffusion constant to give a permeability constant k .

The equation becomes

$$dS/dt = kA(C_o - C_i), \quad \dots\dots(6)$$

where a positive value of dS/dt indicates penetration into the cell and S = the amount of substance penetrated into the cell, C_i = the concentration of the substance inside the cell, C_o = the concentration of substance outside the cell, A = the area of the cell, k = the permeability constant.

The permeability constant k is thus the number of moles of the substance which cross unit cross-sectional area of the membrane in unit time under unit concentration difference; the unit of area chosen is the square micron (μ^2); of concentration difference, moles per litre; and of time, the second.

Equation (6) is fundamental to all permeability studies and the problem is to apply it to the given experimental conditions and the dependent variable to be measured. Let us choose almost the simplest possible case. The cell, into which a given foreign substance is penetrating, is assumed to be of constant volume, V , and surface area, A , and to be suspended in a large volume of fluid so that the external concentration C_o of the penetrating substance can be considered constant. Such a state of affairs would be given by a plant cell whose cellulose wall was strong enough to resist osmotic forces.

In these circumstances we may write

$$dS/dt = kA(C_o - C_i). \quad \dots\dots 6$$

Since $C_i = S/V$, and since A and V are constant, the equation becomes

$$\frac{dC_i}{dt} = k \frac{A}{V} (C_o - C_i), \quad \dots\dots 7$$

which on integration gives

$$\left. \begin{aligned} \ln \frac{C_o}{C_o - C_i} &= \frac{kAt}{V} \\ e^{-\frac{kAt}{V}} &= \frac{C_o - C_i}{C_o} \end{aligned} \right\} \quad \dots\dots (8)$$

or

This simple treatment brings out an important point, namely, the dependence of the rate of concentration change on the value of A/V . Thus if the rates of change of concentration of a penetrating substance in, first, a single cell of radius 1 cm. and, second, a suspension of cells having a total aggregate volume equal to that of the single cell but individual radii of 1×10^{-3} cm., it will be clear that, other things being equal, the rates will be in the ratio of 1 : 1000. Since in biological systems the latter state of affairs is usually the case, i.e. the A/V ratio is large, it follows that equilibria will be achieved in biological systems very much

more rapidly than in artificial systems, e.g. the collodion sac, in which the A/V ratio is comparatively small, even though the actual permeability constants in the two instances may be of the same order.

Equation (8) indicates that the graph obtained by plotting the logarithm of $(C_0 - C_t)/C_0$ against time will be a straight line passing through the origin with a slope equal to $-kA/V$; knowing A/V , k could thus be obtained. The plotting of a graph in this way would be the most satisfactory method of evaluating k ; however, the value could be obtained from a single value of $(C_0 - C_t)/C_0$ and of t , by substitution in equation (8). In many

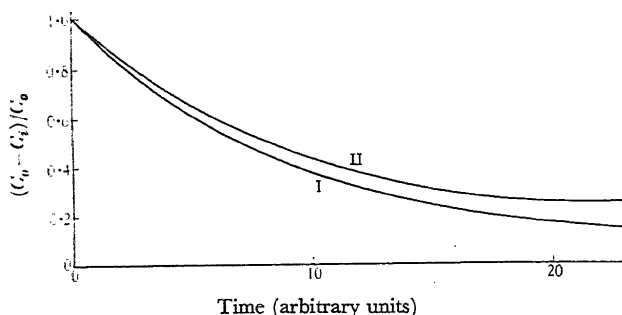


FIG. 12. Curves illustrating the use of equation (8).

experimental techniques it has been considered sufficient to measure a single value of these variables, but instead of using the logarithmic relationship exemplified by equation (8) the value of $(C_0 - C_t)/C_0$ has been used as a quantity directly proportional to the permeability constant. The justification for such a procedure within certain limits will appear from Fig. 12, where the value of $(C_0 - C_t)/C_0$ has been plotted against t , when the relationship between these variables is given by equation (8). The curve, of course, is a logarithmic one, and it is seen that over a portion of its range it is linear, i.e. the logarithm of a number varies linearly with the number itself over a certain range. Hence, so long as values of $(C_0 - C_t)/C_0$ which lie on the linear part of the curve are chosen, it will be justifiable to regard these values when divided by their appropriate time values as being directly proportional to the permeability constant.

Equation (8) indicates that the penetration of the substance will cease when $C_o - C_i$ equals zero; when indirect measures of concentration are used, as for example the volume of the cell, it may happen that the proportionality factor relating the indirect measure to the actual concentration may vary from one set of experimental conditions to another; in this case it may happen that the permeability process will apparently cease when $C_o - C_i$ is not equal to zero, when errors will appear in the determined permeability constants. In Fig. 12 curves I and II demonstrate this point; curve II is identical with curve I, except that the equilibrium position of $C_o - C_i$ is given by 0.1 instead of zero. It will be seen that the error in the value of $(C_o - C_i)/C_o$ introduced by the assumption that the two curves are identical increases the nearer this value is to the equilibrium position, consequently errors due to such changes in the position of apparent equilibrium may be minimised by restricting the measurements to a region of the permeability process as far removed from the equilibrium condition as possible.

The simple relationship between the concentration of the penetrating substance in the cell and time given by equation (8) has been used to demonstrate these points only for the sake of clarity; in actuality, equation (8) would be rarely applicable in permeability studies, but the same considerations which we have raised here would apply to any equation of the form $kt = f(C_i)$. Thus it will be seen that the rate of penetration of water into the erythrocyte may be expressed in the form $k \cdot \Delta t = \text{constant} \times 1/C^2$, where C is the concentration of osmotically active material in the cell. By plotting t against C it will be found that a portion of the curve will be almost linear, and hence over this range the time required for a given value of C to be achieved will be approximately inversely proportional to the permeability constant for water. Similarly, the remarks on changes in equilibrium conditions will apply with equal force to this case.

The condition in which water penetrates the egg of *Arbacia* is more complicated, since here both the volume and area of the cell vary. The rate of penetration is, in this instance, proportional to the difference of osmotic pressure; hence the differential equation will be

$$dV/dt = kA(P - P_{ex}), \quad \dots\dots(9)$$

where V = volume of cell,

P = the osmotic pressure in the cell at any moment,

$P_{\text{ex.}}$ = the osmotic pressure of the solution outside the cell, assumed to be constant,

k = permeability constant defined as number of μ^3 of H_2O which pass μ^2 of membrane with one atmosphere osmotic pressure difference in one second.

Since P will be given by $P_0 V_0/V$, where P_0 and V_0 are the initial osmotic pressure and volume of the cell contents, and the area of the cell, A , will be given by $(36\pi)^{\frac{1}{3}} V^{\frac{2}{3}}$ (the egg being approximately a sphere), the differential equation becomes

$$\frac{dV}{dt} = k(36\pi)^{\frac{1}{3}} V^{\frac{2}{3}} \left(\frac{P_0 V_0}{V} - P_{\text{ex.}} \right). \quad \dots\dots(10)$$

This, on integration, gives an equation connecting the volume of the cell and the time, of the form

$$kt = \ln f(V) + \tan^{-1} f'(V) + f''(V) \quad \dots\dots(11)$$

(Lucké *et al.* 1931; also Northrop, 1927).

If it is desired to measure the permeability constant of water penetrating into the erythrocyte, and the variables measured are the time and the degree of haemolysis, as opposed to time and volume in the case of the *Arbacia* egg, the following simple calculation can be made (Jacobs, 1932).

The cells are placed in distilled water and the bulk of the water is large compared with that of the cells. The fundamental differential equation becomes

$$\frac{dV}{dt} = kAC, \quad \dots\dots(12)$$

where V = the volume of the fluid contents of the cells,

A = the area of the cells,

C = the concentration of osmotically active material in the cells.

If V_0 and C_0 are the initial values of C and V , their values at any time will be given by

$$V = V_0 \frac{C_0}{C}. \quad \dots\dots(13)$$

Hence
$$-\frac{C_0 I_0}{C^2} \frac{dC}{dt} = kAC. \quad \dots\dots (14)$$

Owing to the fact that the area of the erythrocyte does not increase appreciably when the cell increases in volume, A may be considered constant, so that equation (14) may be integrated to give

$$kAt = \frac{C_0 I_0}{2} \left(\frac{1}{C^2} - \frac{1}{C_0^2} \right). \quad \dots\dots (15)$$

This equation connects the time and the concentration of substances in the cell, which, as water penetrates the latter, decreases. If the time of haemolysis is being measured, then C_h will represent the concentration of substances in that group of cells which just haemolyse when the external concentration is equal to this value. If t_h represents the time required to reach the degree of haemolysis corresponding to a value of C_h , equation (15) becomes

$$kAt_h = \frac{C_0 I_0}{2} \left(\frac{1}{C_h^2} - \frac{1}{C_0^2} \right). \quad \dots\dots (16)$$

Thus, if the time for 75% haemolysis is measured, and by fragility studies the value of C_h appropriate to this degree of haemolysis is determined, the value of k may be determined by substitution in equation (16).

We have seen in Chapter III that, if a cell is suspended in a solution of a penetrating substance, the penetration of this substance will cause a difference of osmotic pressure, so that water tends to pass into the cell unless the volume of the latter can be maintained constant; in other words dC/dt will depend not only on dS/dt but also on dw/dt . The equations by which the permeability constant of the penetrating substance may be derived are complicated and their derivation cannot be entered into fully here.

The fundamental differential equations will be as follows:

$$\frac{dS}{dt} = k_1 A \left(C_s - \frac{S}{V} \right), \quad \dots\dots (17)$$

$$\frac{dV}{dt} = k_2 A \left(\frac{a-s}{V} - C_m - C_s \right), \quad \dots\dots (18)$$

where C_s = the external concentration of the penetrating substance,

C_m = the concentration of the external medium before the penetrating substance is added,

k_1 = the permeability constant for the penetrating substance,

k_2 = the permeability constant for water,

a = the amount of osmotically active material originally in the cell,

A = the area of the cell,

V = the volume of the cell.

A general solution of these simultaneous equations has not proved practicable; Jacobs (1934), by mainly numerical methods, has solved them, in the case of the erythrocyte (assuming that the area of the cell is constant), in an exceedingly satisfactory manner. Table III, which has been constructed by Jacobs, enables us to

TABLE III. RATIOS OF THE TIMES THEORETICALLY REQUIRED TO REACH A GIVEN VOLUME IN AN ISOSMOTIC SOLUTION OF A PENETRATING SUBSTANCE AND IN WATER, RESPECTIVELY, FOR DIFFERENT VALUES OF k_1/k_2

$\frac{V}{V_0}$	∞	100	20	10	7	5	4	3	2
1.005	1.00	2.40	4.80	6.80	8.00	9.40	10.40	12.00	14.00
1.01	1.00	1.90	3.50	4.90	5.80	6.80	7.50	8.70	10.60
1.05	1.00	1.24	1.88	2.48	2.90	3.36	3.74	4.24	5.14
1.10	1.00	1.10	1.49	1.87	2.15	2.47	2.72	3.08	3.71
1.25	1.00	1.05	1.23	1.44	1.59	1.78	1.95	2.18	2.60
1.50	1.00	1.03	1.13	1.25	1.35	1.48	1.59	1.75	2.06
1.70	1.00	1.02	1.10	1.20	1.28	1.39	1.48	1.62	1.88
2.00	1.00	1.02	1.08	1.16	1.23	1.32	1.39	1.51	1.75
1	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{4}$	$\frac{1}{5}$	$\frac{1}{7}$	$\frac{1}{10}$	$\frac{1}{15}$	$\frac{1}{20}$	$\frac{1}{30}$
20.80	29.20	36.00	41.6	47.00	56.00	68.0	96.0	124.0	190.0
14.90	21.20	26.10	30.00	34.00	40.60	49.0	70.0	90.0	134.0
7.20	10.28	12.72	14.96	16.80	20.30	24.80	37.60	48.0	72.0
5.18	7.43	9.23	10.90	12.33	15.07	18.70	29.70	37.60	55.5
3.61	5.19	6.55	7.80	8.89	11.18	14.40	22.50	28.0	40.0
2.83	4.12	5.26	6.36	7.38	9.47	12.50	19.0	24.0	34.6
2.57	3.77	4.86	5.92	6.93	8.98	12.00	18.0	23.0	33.0
2.37	3.49	4.54	5.58	6.58	8.61	11.60	17.6	22.6	32.7

read off directly the value of the ratio k_1/k_2 when the ratio of the times required to reach a given volume, V (initial volume of cell equals unity), in the solution of the penetrating solute and in water alone are known. k_2 , the permeability of the erythrocyte to water, may be determined by use of equation (16), whence k_1 may

be obtained. In actuality it is found convenient to measure the time required for a certain degree of haemolysis to occur, as opposed to the measurement of a volume change; in this case the concentration of salts in a solution which causes the degree of haemolysis measured must be known; from this concentration the haemolytic volume of the group of cells which just brings the degree of haemolysis to 75%, may be calculated; the value adopted for 75% haemolysis is 1.70. Hence to obtain k_1/k_2 from the values of the times for 75% haemolysis in the solution of the solute and in pure water, we choose the row corresponding to the value of V equal to 1.70 and note the column in which the value of the ratio t (solute)/ t (water) occurs; the figure at the head of the column gives the value of k_1/k_2 .

The units chosen for concentration, volume and surface area in the derivation of this table are arbitrary and the value of k_2 must be multiplied by the initial cell volume in cubic micra and divided by the initial cell surface in square micra and by the osmotic pressure of the external isosmotic solution in atmospheres. The units will then be in μ^3 of water per μ^2 per atm. per min. The values of k_1 must be multiplied by the osmotically effective initial volume of the cell and divided by the initial cell surface. The units will then be in moles per μ^2 per mole per litre per min.*

It is to be noted that this treatment applies to the case of erythrocytes suspended in an isosmotic solution of the penetrating substance; in the case of the sea-urchin egg it is advisable to add the penetrating substance to the sea water surrounding the egg. In these circumstances the egg will shrink first, and then increase in volume until equilibrium is established at the initial volume. Jacobs (1933) has constructed a chart, obtained by the numerical solution of the appropriate simultaneous differential equations, which we reproduce in Fig. 12*a*; from a knowledge of the initial and minimum volumes of the egg and the time required for the attainment of the minimum volume the values of both k_1 and k_2 may be read off directly. The units employed for the construction of this chart differ from those generally used and conversion factors must be employed. These have been provided by Jacobs (1933) and are given in Table III*a*.

* Jacobs generally uses the minute as the unit of time, whereas we have used the second.

If the egg is suspended in an isotonic solution consisting of both non-penetrating salts and of the penetrating substance, it is clear that a calculation which is based on the determination of a minimum volume is of no use, since the only volume change to

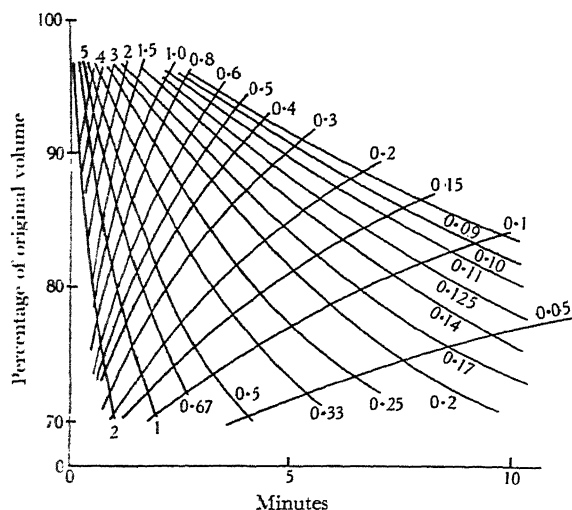


FIG. 12a Chart for calculation of permeability constants for *Arbacia* eggs (after Jacobs, 1933).

TABLE IIIa. CONVERSION FACTORS FOR PERMEABILITY CONSTANTS

k_1 is in gm. mols per μ^2 per min. per gram mole per litre concentration difference, and k_2 is μ^5 per μ^2 per min. per atmosphere osmotic pressure difference

Cell volume in μ^3	Factor for $k_1 (\times 10^{15})$ (solute)	Factor for k_2 (water)
180,000	11.68	0.50
185,000	11.78	0.51
190,000	11.89	0.51
195,000	11.99	0.51
200,000	12.09	0.52
205,000	12.19	0.52
210,000	12.29	0.53
215,000	12.39	0.53
220,000	12.48	0.54
225,000	12.58	0.54
230,000	12.67	0.54
235,000	12.76	0.55
240,000	12.85	0.55

occur will be a continuous swelling. Hunter (1939) has adapted Jacobs' equations for this special case, however.

A final point to remember, in regard to the equations discussed in this chapter, is that the volume of the cell, denoted by V , should be equated to the volume of solution contained by the cell, and this will differ from the geometrical volume by the volume of solid matter in it; in the case of the erythrocyte this represents about 30%, of the total volume of the cell.

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by method (1), since the movements of the larger molecules will be much more sluggish. But for large molecules diffusing in a medium of smaller molecules and for ions in water, method (2) probably predominates, since movements of the small molecules can provide holes more rapidly than the large molecule can do so by virtue of its own kinetic energy.

In the case of polar liquids, such as water, glycerol and sulphuric acid, chemical bonds (usually hydrogen or hydroxyl bonds—see Fowler & Bernal, 1933) also restrain the movements of molecules. For example, if we compare these molecules with other molecules which do not form such bonds, e.g. H_2O with H_2S , H_2Se , or NH_3 which do not readily form these bonds, or perchloric acid HClO_4 which has only one hydrogen atom available for bond formation, with sulphuric acid H_2SO_4 (which has two bond-forming hydrogen atoms), we find that the substance which forms hydrogen bonds most readily has the higher boiling point and lower vapour pressure (because molecules cannot so easily diffuse into the vapour phase, and the higher viscosity because molecules are restrained from diffusing in the interior of the liquid by the hydrogen bonds). Thus, the boiling point of water is 100°C . compared with -62°C . for H_2S , -42°C . for H_2Se , and -33°C . for NH_3 . So we find that in a liquid such as water, diffusion is retarded both by Van der Waals' forces and by chemical bond forces. But the basic two modes of diffusion referred to above remain the two fundamental types: the chief effect of hydrogen-bond formation is to make diffusion more difficult, in so far that sufficient kinetic energy is required to overcome not only the Van der Waals' forces but also the chemical bond forces.

Where the total energy required to break all of the forces tending to restrict free diffusion is large, it frequently happens that part only of a molecule diffuses at a time. For example, when a glycerol ($\text{CH}_2\text{OH}.\text{CHOH}.\text{CH}_2\text{OH}$) molecule evaporates from a water surface all these forces are broken simultaneously, so that the energy required is of the order of 20,000 calories per gram mol. But the activation energy for diffusion of glycerol in water is only about 4000 calories. This means that in water the molecule diffuses by relatively small amounts at any one time, making small movements and rotations which involve overcoming part only of the restraining forces.

Fick 1855 deduced on theoretical grounds that equation (19) should hold:

$$\frac{\partial s}{\partial t} = -D \frac{\partial c}{\partial x}, \quad \dots\dots(19)$$

where ∂s is the amount of substance diffusing across an area of 1 cm.² in time ∂t under a concentration gradient $\partial c / \partial x$; D gives the amount of substance diffusing across an area of 1 cm.² per unit time when $\partial c / \partial x = 1$.

It is not generally appreciated that Fick assumed that the resistance to diffusion is continuous, due e.g. to a medium of particle size which is negligible compared with that of the diffusing molecule. In any real liquid, however, most of the molecules whose diffusion is studied are of the same order of magnitude as the solvent molecules, and, for reasons which were presented above, diffusion is intermittent in character. Fick's equation, therefore, on its present theoretical foundation, is of approximate accuracy only. It is fortunately the case that if we measure diffusion across a solvent layer of thickness very large compared with the diameter of the diffusing molecules, Fick's equation holds within very precise limits. When, however, we are dealing with a thin layer or membrane of thickness of the order of 10 m μ , as is frequently necessary in biology, Fick's equation is only roughly true, and it is necessary to take into account some of the details of the structure of the membrane if a really accurate treatment is desired. This, however, is very difficult to do.

Fick's constant D is a measure of the resistance offered to free diffusion. In water the values of D are thousands of times less than in a gas such as oxygen at normal temperature and pressure, due to the resistance offered by the Van der Waals' and chemical bond forces acting between adjacent molecules. Thover (1910) applied Maxwell's equation to diffusion in liquids; he found that the expression

$$DM^{\frac{1}{2}} = \text{constant} \quad \dots\dots(20)$$

is very roughly true for molecules up to the size of hexoses diffusing in such solvents as water and alcohol. Öholm (1912) confirmed this result. Table IV shows some typical results for water. The value of the constant in equation (20) is much smaller for diffusion in water than in gases.

TABLE IV. VALUES OF $DM^{\frac{1}{2}}$ FOR DIFFUSION IN WATER

Substance	$D \cdot 10^5$	M	$DM^{\frac{1}{2}} \cdot 10^5$
Methyl alcohol	1.28	32	7.2
Acetamide	0.96	59	7.4
Urea	0.94	60	7.3
Benzyl alcohol	0.77	74	6.6
Amyl alcohol	0.69	88	6.5
Glycerol	0.72	92	6.9
Chloral hydrate	0.58	165.9	7.5
Glucose	0.52	180	7.0

Einstein (1905, 1906) has derived an equation relating D to the viscosity, η , of the diffusion medium and to the radius α of the diffusing molecule:

$$D = \frac{RT}{N} \cdot \frac{1}{6\pi\alpha\eta}, \quad \dots\dots 21$$

where N is Avogadro's number and R is the gas constant. The temperature coefficient of D is given by

$$Q_{10} = \frac{D_{T+10}}{D_T} = \frac{T+10}{T} \cdot \frac{\eta_T}{\eta_{T+10}}, \quad \dots\dots 22$$

i.e. for very dilute solutions, which have the same viscosity, the Q_{10} should be the same for all molecules. Einstein's equation was based on the assumption that the solvent molecules are negligibly small compared with the solute molecules which are under investigation. This is true for colloidal particles, whose behaviour seems to be fairly accurately described by this equation. But this assumption is not complied with in the case of smaller molecules such as urea and glycerol, which may be the same size or even smaller than the solvent molecules. In view of this it is not surprising to find that if values of α , the molecular radius, are calculated for small molecules from equation (22), using the experimental values of D for diffusion in water, the values of α often are impossibly small, and that the Q_{10} of D , instead of being constant, varies from 1.28 (for $D=2.4$) to 1.48 (for $D=0.2$): i.e. the more slowly diffusing molecules have the higher Q_{10} values. In view of the facts that Einstein's equation is theoretically inapplicable to small molecules, and that if applied as an empirical equation it gives incorrect results, it is somewhat to be regretted that it has been studied to the exclusion of the more accurate equation of Thoevert. As Öholm (1912) pointed out, Einstein's equation is accurate only under rather special and limited circumstances.

Danielli (Appendix A) has made a crude attempt to obtain an equation which takes some account of the structure of the medium in which diffusion occurs. He obtained the following equations:

$$DM^{\frac{1}{2}} e^{\frac{\mu_e}{RT}} = \lambda r \phi \sqrt{\frac{RT}{2\pi}}, \quad \dots\dots(23)$$

$$(Q_{10})_D = \sqrt{\frac{T+10}{T}} e^{\frac{\mu_e}{RT} \left(\frac{10}{T+10} \right)}. \quad \dots\dots(24)$$

And, substituting for $e^{\frac{\mu_e}{RT}}$ from (24), at constant temperature and constant ϕ :

$$DM^{\frac{1}{2}} Q_{10}^{(T+10)/10} = \text{constant}. \quad \dots\dots(25)$$

These are approximate equations only, and, within their limits, fit the facts well. μ_e is the activation energy of diffusion, and ϕ is the probability that a molecule, having the minimum kinetic energy μ_e necessary for diffusion, will also have the correct orientation, etc. to enable it to diffuse. The variation in μ_e and ϕ provides for the difference between the rates of diffusion of the same molecule in different solvents, and for the variation in Q_{10} values with different molecules in the same solvent. In a liquid of low viscosity the μ values do not vary greatly from molecule to molecule, and the Q_{10} values therefore do not vary much. Hence we see from equation (25) that $DM^{\frac{1}{2}}$ should be approximately constant, i.e. we obtain a possible theoretical basis for Thover's equation. For diffusion through very viscous liquids and solids, on the other hand, the Q_{10} 's will vary greatly, and $DM^{\frac{1}{2}}$ will not be constant. This is useful for diagnostic purposes, for wherever we meet diffusion in (liquid + membrane) systems characterised by a low Q_{10} and obeying the relationship $DM^{\frac{1}{2}} = \text{constant}$ we may be fairly sure we are dealing with diffusion in water or some other liquid of comparatively low viscosity.

Now, lastly, let us consider the process of diffusion through a thin layer of fatty (hydrocarbon) material lying between two aqueous layers. First consider the qualitative aspects of the problem. Diffusion through the water layers will be comparatively rapid and the resistance due to the water may in many cases be neglected by comparison with that due to the fatty layer. When a molecule reaches the fatty layer, there may be a discontinuity; before it can enter into the fatty layer it must acquire

sufficient kinetic energy both to overcome the forces holding it to water and to make a hole in the fatty layer. For a molecule such as sugar, which forms many hydrogen bonds, the amount of energy required is large; and as a large amount of kinetic energy is seldom available the process of diffusion into the oil layer is comparatively slow. On the other hand, for a molecule such as benzene, which forms no hydrogen bonds with water, diffusion into the oil layer requires only a small amount of kinetic energy and will therefore be rapid. In diffusing across the oil layer, the resistance encountered will be almost entirely due to Van der Waals' forces, but this may be very considerable where the oil is composed of large molecules such as sterols and lipins. When the diffusing molecule has crossed the oil layer it has finally to diffuse across the oil-water interface into the water. For a molecule like glycerol this will be comparatively easy, since when it comes to the interface it will form hydrogen bonds with the water and virtually become part of the water, and will be able to diffuse away into the interior of the water layer by the same easy stages as through water in bulk, with little opposition from surface-tension forces. But with a molecule like benzene the process may be more difficult. Benzene forms no hydrogen bonds with water, and therefore cannot become incorporated in the water layer. Consequently to penetrate into the water it must have sufficient kinetic energy to remove itself completely from the fatty layer and penetrate into the water. If the benzene molecule only partially removes itself from the oil layer, it will simply form an excrescence from the oil layer, increasing the surface area of the oil layer, and will immediately be pushed back from the water by surface-tension forces. Since a rather large hole must be made in the water by the penetrating benzene molecule, the energy required is rather large and the rate of passage rather low.

The quantitative treatment of diffusion across such an oil layer presents many difficulties. If one assumes that Fick's Law applies to diffusion across the oil layer, it is possible to use the same equations as were used in the study of the diffusion of heat. Such studies have been made in connection with the diffusion of hydrogen across balloon membranes, but are not of much use for biological studies. F.J. Turton has made a study in a form
o biological studies. He finds that for molecules

which penetrate the oil layer very slowly, under certain conditions (see Appendix A), the permeability is given by the equation $P = a^2$. a is the constant for the rate of diffusion of molecules from water into the oil layer, which is given by aC , where C is the concentration in the water layer. Turton has not published this study, as it has no special mathematical merit and is inferior both in accuracy and in convenience of use to his second study made on a model proposed by Danielli (Appendix A), which takes some account of the structure of the oil layer, instead of using the inaccurate assumption that Fick's Law holds for diffusion across it. Consider Fig. 13*a* and *b*. Fig. 13*a* shows the form of

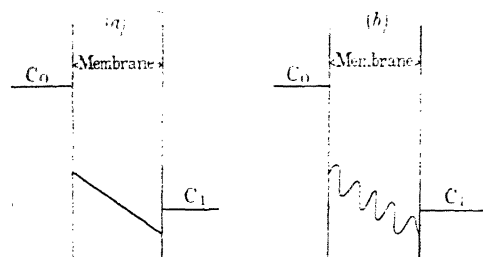


FIG. 13. Concentration drops across a small element of volume of a membrane: *a*, membrane without structure, *b*, membrane with structure.

concentration gradient met with if Fick's Law is assumed to be correct; Fig. 13*b* shows the concentration gradient obtained for a small element of membrane volume, assuming an approximation to the structure of the interior of the oil layer. Any individual molecule penetrating the membrane meets a number of potential energy barriers, due to the fact that the molecules composing the membrane present a series of more or less discrete areas of intermolecular action, e.g. the successive CH_2 groups in a hydrocarbon chain. Each of these barriers can only be crossed if the diffusing molecule has sufficient kinetic energy. If the number of these potential energy maxima is n , the constant for diffusion across these maxima is e , and b is the constant for diffusion across the oil-water interface in the direction oil \rightarrow water, then the permeability of the membrane is given by

$$P = ae$$

For many rapidly diffusing molecules $2e$ is negligible compared with πb , and this simplifies to

$$P = \frac{a}{b} \cdot \frac{c}{n} \quad \dots\dots 27$$

For many slowly penetrating molecules $2e$ is much greater than πb , and we obtain from 26

$$P = \frac{a}{2}, \quad \dots\dots 28$$

which is the same equation as was obtained in Turton's first treatment. Now this case, where $2e$ is much greater than πb , corresponds to the physical condition in which diffusion across the oil-water interface in the direction water \rightarrow oil is so much slower than diffusion across the rest of the oil layer that the resistance due to the rest of the oil layer may be neglected, i.e. the assumptions made as to the form of the concentration gradient in the interior of the membrane are in this case of no importance. Consequently, one can predict that in this case the two treatments should, as they do, give the same result. For rapidly diffusing molecules the first treatment gives a result which is much more complicated, and, for thin membranes, probably less accurate than the second treatment.

From these equations it is possible to calculate the permeability of e.g. a thin layer of olive oil. The results of such a calculation are given in Table XXII, in the chapter on non-electrolytes. It will be seen that the permeability of such a layer closely resembles that of typical cell membranes.

All the equations which have been given dealing with the permeability of a thin oil layer are approximate only. This is because they take account of the structure of the oil layer in an approximate way only. It is at this point that we reach a limit to the possibility of developing a more refined theory. This limit is imposed by the fact that diffusion theory has not yet coped with the effect of the structure of the solvent. The most recent papers on this subject (see e.g. Lennard-Jones *et al.* 1939; Eyring, 1936) suggest that there is little prospect of an immediate advance of a type which will be useful for b

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CHAPTER VI

THE STRUCTURE OF THE PLASMA MEMBRANE

By J. F. DANIELLI and H. DAVSON

IN this chapter will be presented a brief review of evidence derived from sources other than permeability studies, relating to the possible structure of the membrane responsible for the selective permeability of the cell. In the final chapter the results of permeability studies will be invoked to see to what extent the theory of membrane structure conforms with the selective powers exhibited by the cells in respect to the penetration of different substances.

The cell membrane, with which permeability studies are mainly concerned, is extremely thin, so that it cannot be differentiated from the protoplasm which it surrounds by microscopic means: in fact its presence is initially inferred from that selective power of the cell surface itself by which certain substances are excluded from entering and others are allowed to pass through with greater or less ease, and also from the fact that if the surface of the cell is punctured or broken the cell contents flow from the aperture to mix freely with the surrounding aqueous medium, and foreign substances then have free access to the interior of the cell. Possible exceptions to this latter generalisation are the claim that the erythrocyte may be cut into halves by micro-dissection without the haemoglobin escaping, and also the observation quoted by Pfeffer (1877) that the root hairs of *Hydrocharis* may be crushed to give small droplets of protoplasm which behave osmotically as individual cells. But in both instances it has yet to be proved that the actual continuity of the membrane of the parent cell was broken, and it is quite possible that the cell divides up as in Fig. 14.

It should be noted that many cells have more than one membrane: thus plant cells are surrounded by a comparatively thick cellulose wall easily distinguished under the microscope: fertilised eggs are frequently surrounded by a tough "fertilisation membrane", and the unfertilised eggs of some species, e.g. *Arbacia punctulata*, are surrounded by a thick layer of jelly. Where investigated it usually has been found that these structures

external to the true cell membrane are highly permeable and exhibit few or no special selective powers in respect to the penetration of various molecules; their chief role is probably a mechanical one and they may be stripped off the cell without significant alteration of its permeability characteristics.

The Lipoid Layer Theory. Overton found that fatty substances readily penetrate this membrane, whereas substances which are insoluble in fats penetrate very slowly, if at all. This led him to

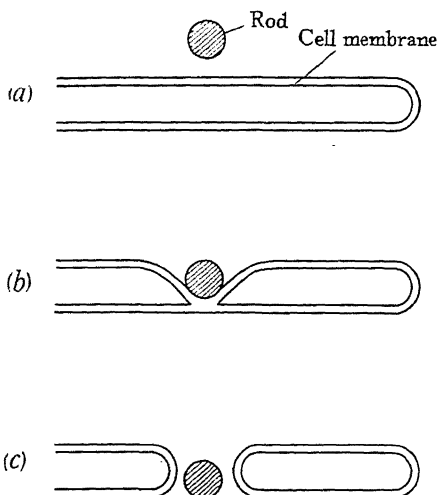


FIG. 14. Production of protoplasmic droplets by crushing root hairs with a rod, so as to maintain continuity of the membrane.

suggest that the membrane is composed of lipoid or fatty molecules (1895, etc.). Actually any molecules made up mainly of CH_2 or CH groups would have the same solvent powers, so that steroids and phospholipids must be considered in addition to true fats and fatty acids. Supporting this suggestion of Overton's is the work of Gorter & Grendel (1925), Chambers (1935), Dawson & Belkin (1929), Mudd & Mudd (1931), Schmitt *et al.* (1938), and Waugh & Schmitt (1940).

Gorter & Grendel found that the total lipoid of the erythrocytes of several different species, when spread in a monolayer, occupied an area just twice the surface area of the erythrocytes. They therefore suggested that this lipoid was arranged at the

TURE OF THE PLASMA MEMBRANE

cell surface in a bimolecular leaflet, with the hydrated polar groups at the interfaces of the film, and the hydrocarbon portions of the molecules making up the interior of the membrane (Fig. 15). Any alternative arrangement of the lipid molecules would be unstable. The lipid molecules are, according to this theory, arranged radially.

Schmitt *et al.* 1936, 1938 examined the birefringence of the red cell ghost with polarised light, and also came to the conclusion

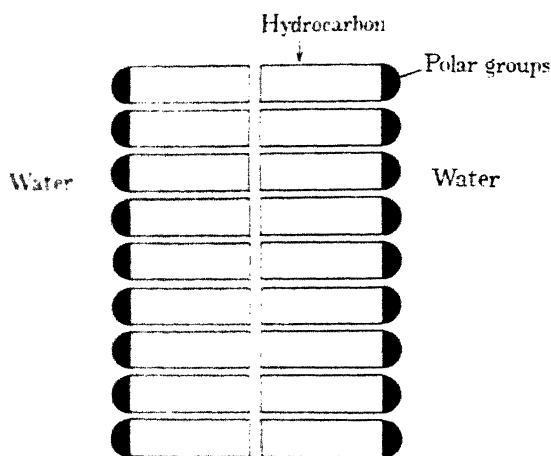


FIG. 15. Structure of red cell membrane after Gorter & Grendel.

that the cell membrane contains radially arranged lipid molecules, and may be no more than a few molecules thick. There are great difficulties, as these authors point out, in the interpretation of polarised light studies, so that at present this observation must be accepted with caution.

The wetting properties of cells studied by Mudd & Mudd, Dawson & Belkin and Chambers are of great interest. Erythrocytes pass readily into the interior of an oil layer, and so must be preferentially wet by oils. Leucocytes, however, are preferentially wet by water. Other cells have been studied by bringing small oil droplets in contact with the cell surface. Globules of oil adhere to the surface of *Amoeba dubia*, forming a cap. Paraffins present in the oil of such a cap will narcotise *Amoebae*, even in which have no narcotic action when the oil is

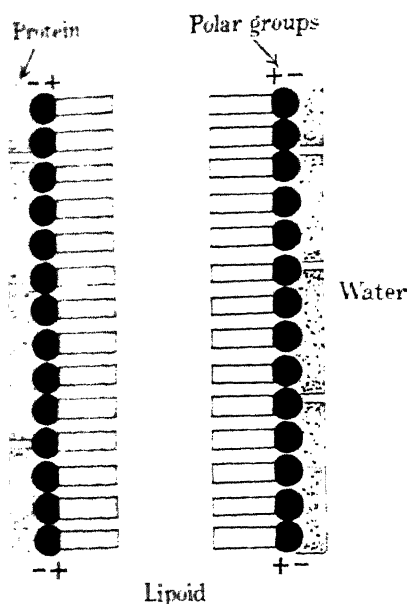
injected into the interior of the cell; so evidently the membrane which is wetted by oil is one fundamental to the activity of the cell (Marsland, 1933). When naked *Arbacia* eggs are used, the oil droplet, if it wets the cell, snaps into the interior. If two such droplets are made to adhere in this way to a single cell, and the sea water stirred gently, the drops show movement relative to one another, so that evidently in this case the membrane is not only fatty in character, but also liquid, i.e. the molecules composing it are not fixed rigidly in space but have the power to slide over one another (Kopac & Chambers, 1937).

The work of Höber (1910, 1912), Fricke (1925), McClendon (1926), Cole (1937), etc. on the electrical impedance of cell membranes to high-frequency alternating currents shows that whereas the cell contents have a fairly high conductivity, the cell membrane is of very low conductivity. This may be considered to support the lipid theory, for it is difficult to see how a thin, and sometimes liquid, layer of protein can have a low conductivity, but a similar layer of lipid will of necessity be of low conductivity, since ions enter a lipid layer with great difficulty.

The Protein Theory. An alternative hypothesis, that proteins are the chief constituent of cell membranes, has also been considered by many authors. Its chief attraction is that the membranes of many cells appear, to qualitative observation, to have the properties of a sieve. This sieve-like character, it was thought, could best be provided by the polypeptide chains of proteins. So far, however, it has not proved possible to obtain protein membranes which sufficiently resemble the cell membrane for this structure to be plausible. Harvey (1912) experimented with protein membranes formed by an adsorption process, finding that NaOH and NH_4OH penetrate equally rapidly, whereas NaOH enters cells much less rapidly than NH_4OH . Collander (1927) prepared membranes by fixing gelatine with formaldehyde; these membranes showed no preferential permeability to lipid-soluble molecules.

Recent Developments. A fresh line of evidence was obtained from the work of Harvey, Cole and their collaborators on the tension at the surface of cells. If a sea-urchin egg is centrifuged, the oil globules (lighter than the cell fluid) collect at the centripetal end of the egg, and the yolk granules (heavier than the cell fluid) collect at the centrifugal end. When thus collected at the two

of the egg, the oil and yolk under centrifugal force exert forces which tend to elongate the egg into a cylinder, and when the length of this cylinder reaches π times its diameter it becomes unstable and divides into two almost equal spheres (Harvey, 1931). The process can be watched in the centrifuge microscope (Harvey, 1932), and the centrifugal force just sufficient to cause division



cell membrane (Danielli) showing the general pattern.

into halves determined. Then the centrifugal force may be equated to the tension round the surface of the elongated cylinder, and thus the tension at the surface of the cell may be calculated. For the unfertilised eggs of *Arbacia*, Harvey found a tension of 0.2 dyne per cm. A second method, due to K.S. Cole (1932), by which the tension is determined from the force necessary to flatten the initially spherical egg to varying degrees, gave a value of 0.08 dyne per cm. Other methods, applied to this and to other cells, have shown that in all cases the tension is very low. Since no lipids are present in the plasma membrane, which, at the pH of living cells, have not only a low

solubility in water like the plasma membrane, but also a tension against the aqueous phase as low as 0.1 dyne per cm., it became evident that some substance other than lipoids must be present in the plasma membrane. Harvey & Shapiro (1934) then showed that these very low tensions are also found at the surface of intracellular oil droplets. On analysis of the cause of this low tension, it was found that the intracellular proteins were responsible (Danielli & Harvey, 1934). Subsequent investigation

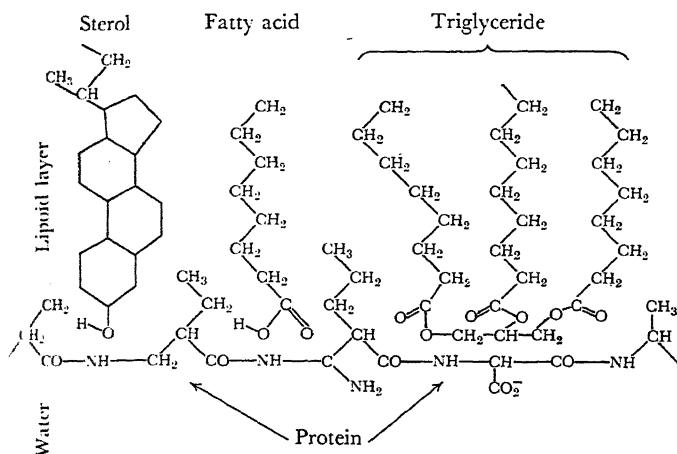


FIG. 16 b. A detail of the structure of the cell membrane (Danielli). The polar groups of the protein are in roughly the same plane as those of the fatty molecules, and the hydrocarbon parts of the protein extend into the fatty layer.

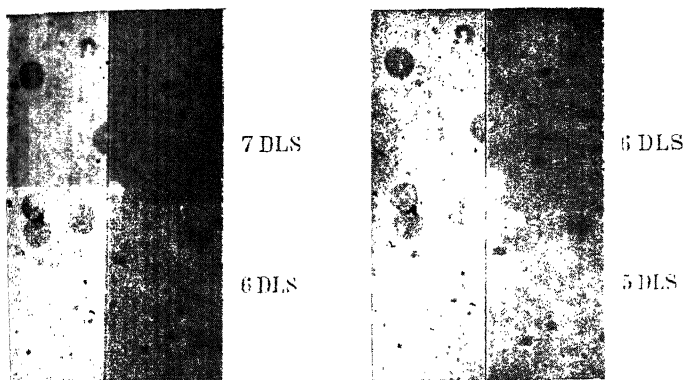
of a large number of proteins showed that many proteins can produce these low tensions (Danielli, 1938). Since proteins have such a large surface activity, it necessarily follows that any lipid surfaces in contact with a protein solution, even if as dilute as one part in 10^7 or more, will be covered with protein, and have a low tension at their surface. This conclusion applies equally to lipid membranes, intracellular oil globules, serum fat particles, etc. Thus the advocates of the presence of a fatty membrane at the surface of protoplasm are forced to accept the simultaneous presence of adsorbed films of protein molecules arranged as in Fig. 16. In the case of the erythrocyte, Schmitt *et al.* have shown

from optical studies that proteins are arranged tangentially on the cell surface, as is demanded by the surface chemistry of proteins.

The properties of this complex membrane are probably not to be regarded as a simple juxtaposition of the properties of the proteins and lipoids. Complex formation between the proteins and lipoids at the cell surface probably occurs to a greater or less extent (*vide e.g.* Schulman & Rideal, 1937), and the investigation of these complexes is a matter of fundamental interest from the point of view of permeability studies.

Thickness and Physical State of the Plasma Membrane. There is no adequate evidence of the thickness of the protein layers, but three lines of evidence are available in the case of the lipoid layer. Electrical impedance studies have shown that the thickness of this layer is of the order of 50 Å.* Gorter & Grendel (1925) found that there is sufficient lipoid in erythrocytes to make a layer two molecules thick in the membrane, corresponding to a thickness of the order of 50 Å. Schmitt *et al.*, from optical studies, conclude that the lipoid layer may be no more than a few molecules thick. An ingenious method of measuring the thickness of the red cell membrane is that of Waugh & Schmitt (1940) with an instrument which they call the Analytical Leptoscope. The essential principle on which this apparatus is based consists of the comparison of the intensities of light reflected from a dried erythrocyte ghost with that from a step film of barium stearate of known thickness. Barium stearate films may be made of graded thicknesses by the simple process of building up successive double layers. In Fig. 17 *a* and *b* are shown photographs of the step films side by side with the erythrocyte preparations, and from these it is clear that the erythrocyte disks are lighter than the 7 D.L. (double layers of stearate film) in one print and darker than the 5 D.L. step on the other; they are fairly close to the 6 D.L. step and may be a trifle lighter (thinner). The results of the thickness determinations of rabbit erythrocyte preparations are shown in Fig. 18, in which the thickness is plotted against the pH of the haemolysing medium, and we note that the thickness has a maximum value of over 200 Å. at pH 6. This corresponds more nearly to the isoelectric point of serum protein than to that of haemoglobin.

* For erythrocytes see Fricke (1925); for leucocytes, Fricke & Curtis (1935); for yeast, Fricke & Curtis (1934); for *Arbacia*, *Asterias* and *Hipponoe* eggs, Cole (1928), Cole & Spencer (1935), Cole & Curtis (1938).



b

FIG. 17. Photographs of erythrocyte preparations and different step films as seen through the leptoscope. The 6 double-layer (DLS) photographs in the two figures are not comparable with each other. The round discs are the erythrocytes, which should be compared with the multilayers on the right of the photographs.

the lower curve represents the thickness of cells extracted with organic solvents and hence probably represents the thickness of protein constituents of the erythrocyte ghost which, of course, are in the membrane or exist as a stroma throughout the cell. The measurements represented by the curves in Fig. 18 were on ghosts allowed to stand just 1 min. in their haemolysing medium; if the time is protracted, a progressive loss of thickness

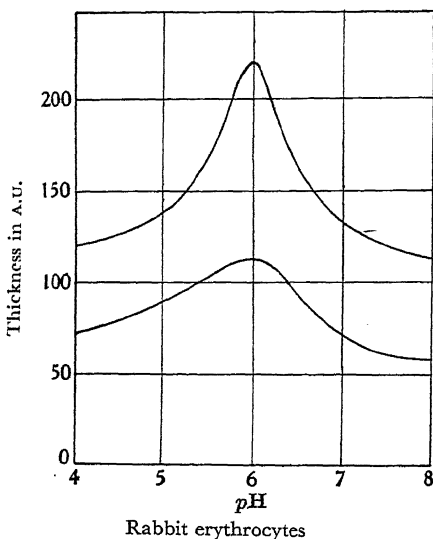


FIG. 18. Corrected thickness of the rabbit erythrocyte envelope as a function of the pH of the haemolysing solution. The upper and lower curves represent respectively the thickness of the whole envelopes and of the envelopes after extraction in organic solvents.

is observed due to the loss of both lipoids and proteins from the ghost; the authors suggest that the cause for this is the low electrolyte content of the medium. Thus, in the case of the erythrocyte, there is a satisfactory upper limit for the thickness of 2-4 molecules, corresponding to a thickness of about 50 Å. For other cells evidence rests solely on impedance studies, and is dependent on assuming a value for the dielectric constant of the lipid layer (Danielli, 1935). Even so, however, it is probably safe, for purposes of calculation, to take 100 Å. as an upper limit for the thickness of the lipid layer.

TURE OF THE PLASMA MEMBRANE

With regard to the physical state of membranes having the thickness and chemical composition discussed above, it is quite clear that the possibilities are limited and, as a result of the work of the last thirty years on surface films, fairly well defined. So far as protoplasm in other regions of the cell is concerned, it has not yet proved possible to obtain any idea of the submicroscopic structures concerned. This, no doubt, is due to the fact that physical science has, as yet, only just begun to develop the theory and technique of dealing with structures composed of micelles. For example, highly significant studies of long-range forces between micelles, acting over distances of between 1 Å. and 1 μ .

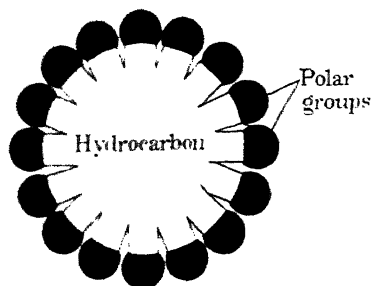


FIG. 19. Sketch of structure of colloid micelle.

have been made by Levine (1939) and by Langmuir (1938*a*); these, however, but touch upon the fringe of the complexities of protoplasm, and it is indeed very probable that the refinement of X-ray technique and of the electron microscope are essential pre-requisites of the analysis of protoplasmic structure. In dealing with the cell membrane, which is the surface layer of protoplasm, we are in a more fortunate position. Insoluble fatty molecules in an aqueous phase can be arranged in two ways only, in micelles and in films. In micelles the minimum thickness is two molecules: individual molecules are arranged with their polar groups in the aqueous phase, their non-polar CH_2 and CH groups oriented towards the centre of the aggregate, forming a minute droplet of more or less liquid hydrocarbon (Fig. 19). In films the minimum possible structure is a layer two molecules thick (Fig. 15), in which the polar groups of each layer are in an oil-water interface, and the hydrocarbon residues of the molecules face inwards to

make a thin film of more or less liquid hydrocarbon. Such films and micelles are probably also stable when more than two molecules thick, but the surface layers must retain the orientation of these two elementary forms. All other forms of arrangement of these molecules are subject to disruption by surface-tension forces (Danielli, 1936).*

It is, of course, not easy to say whether the lipoids composing the plasma membrane will be in the form of a film composed of rigidly oriented molecules held together by Van der Waals' forces of adhesion, i.e. in the form of a solid film, or whether the molecules will be in a less rigid structure which will allow them a certain independence of movement. The probability is that the molecules of the lipoid will be arranged in the latter manner, since in thickness the membrane approximates to two monolayers; monolayers of steroids and of glycerides are rarely solid films in the physiological temperature range (*vide e.g.* Adam, 1938). Proteins may form membranes by cross-linkage of polypeptide chains arranged in bundles, by formation of S—S linkages, for example, such as stabilise wool fibres (Astbury, 1938; Goddard & Michaelis, 1934). Such membranes would have a high degree of stability. They may also form very stable films one molecule thick by adsorption at oil-water interfaces, if the interface is liquid (Danielli, 1938). At an oil-water interface even the globular proteins unroll into thin sheets or long chains, which are oriented so that the hydrocarbon residues of the amino acids are incorporated in the oil layer, whilst the polar groups remain in the aqueous phase. Such films may be liquid or elastic solids. The hydrocarbon residues anchor the molecule to the oil layer, so that when unfolded and adsorbed in this way, the original solubility is completely lost, and the resulting protein film of between 5 and 50 Å. in thickness is quite stable. Globular protein molecules may adsorb in one or more layers on to such an unfolded protein film (Fig. 20), or on to a solid fatty surface, without losing the globular condition (Langmuir, 1938*b*). Such layers, however, probably contribute little to the mechanical strength of the membrane.

* De Jongh & Bonner (1935) have suggested that phospholipins may form bimolecular layers with the polar groups directed inwards. In this special case stability may perhaps be achieved by salt formation between NH_2 and CO_2H of the opposing layers of the film.

We have already seen that the membrane contains both lipoid and protein, so that if the latter is adsorbed on the surface of the lipoid one may expect it to be in the form of the stable films described by Danielli 1938, and it is possible that its presence may impart to the remainder of the membrane a structural solidity which the lipoids alone could not achieve. The presence

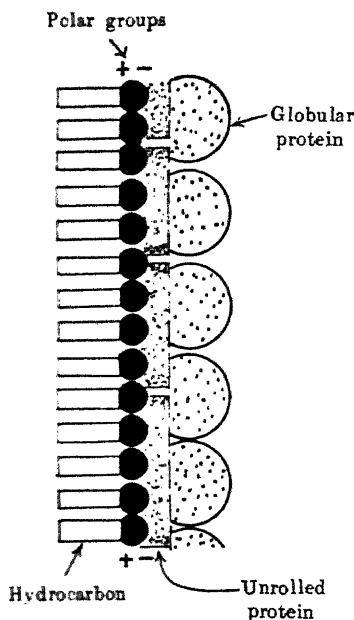


FIG. 20. Sketch of globular protein molecules adsorbed on to a film of unrolled protein molecules previously adsorbed at an oil-water interface.

of a cross-linked meshwork of polypeptide chains, such as one would expect were the adsorbed protein to unfold itself on the cell surface, may perhaps account for the sieve-like properties of some cell membranes, to be described in later chapters.

X-Ray Studies. The plasma membrane of cells is probably too thin for a direct study of its ultra-structure to be made by X-ray diffractometry; however, Schmitt & Palmer (1940) have made a detailed analysis of the X-ray diffraction spectra of lipoids and proteins and of the nerve axon myelin sheath. This sheath is a

thick lipo-protein structure, and it seems clear that it is not the functional membrane which separates the axoplasm of the nerve from its environment, yet it seems that this sheath is built up on a

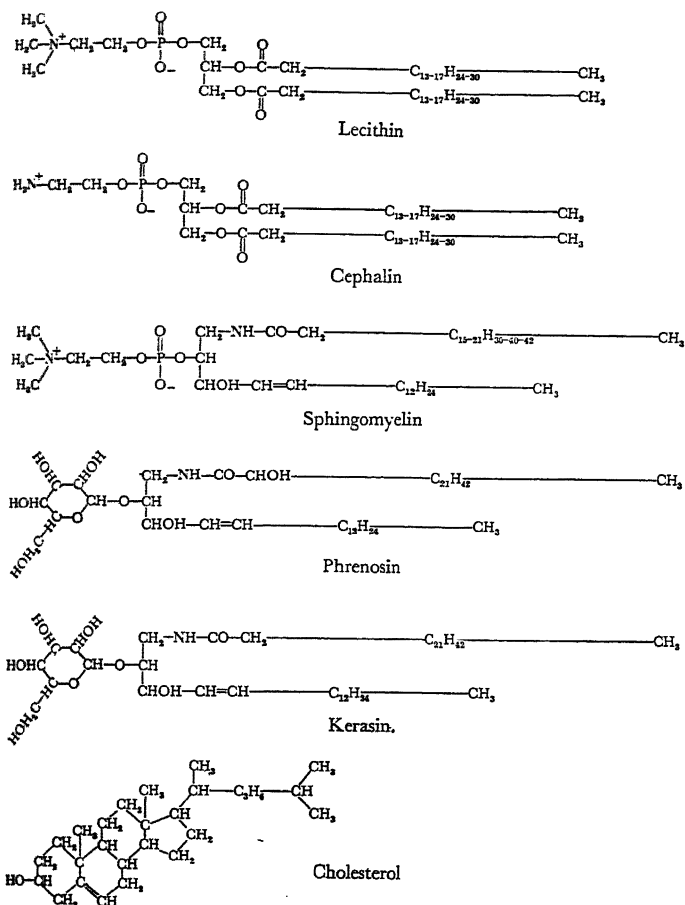


FIG. 21. Stereochemical formulae of certain nerve lipids.

similar plan to that of the thinner selectively permeable membrane of the nerve and other cells (Schmitt & Bear, 1939). For this reason a few of the outstanding points brought out by the work of Schmitt & Palmer may be mentioned. In Fig. 21 are presented

the structural formulae of some of the lipid molecules known to be present in cell membranes, and in Fig. 22 are shown the diffraction spacings of the dry lipids singly and in mixtures and also those for an extract of the lipids from the spinal cord and for the dried and otherwise intact motor nerve root. The small

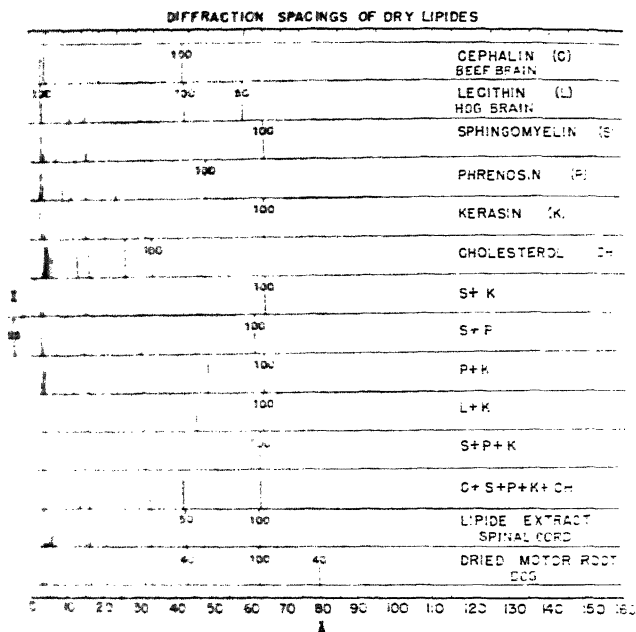


FIG. 22. Spacings plotted horizontally, and visually estimated intensities plotted vertically, of diffractions obtained from pure and mixed lipids and from dry nerve.

spacings to the left indicate the lateral spacing between oriented lipid molecules; the larger ones to the right show the thickness of successive layers of the lipid; it is to be noted that there is a large spacing of about 160 Å. in the dried motor root which is found with the dry lipid mixtures, and this large identity period is the main evidence for assuming that in the nerve sheath protein enters into the structural pattern. Knowing the thickness of the bimolecular leaflets of lipids, the thickness of the protein contribution to the identity period would be about 25 Å., on the

assumption that there are two bimolecular leaflets to each period. In Fig. 23 is shown a diagrammatic representation of the effects of increasing the amount of water with which brain lipids were emulsified on the spacings determined by X-ray methods; the large amounts of water which can be held by an oriented framework of lipoids is indeed surprising and it seems probable that

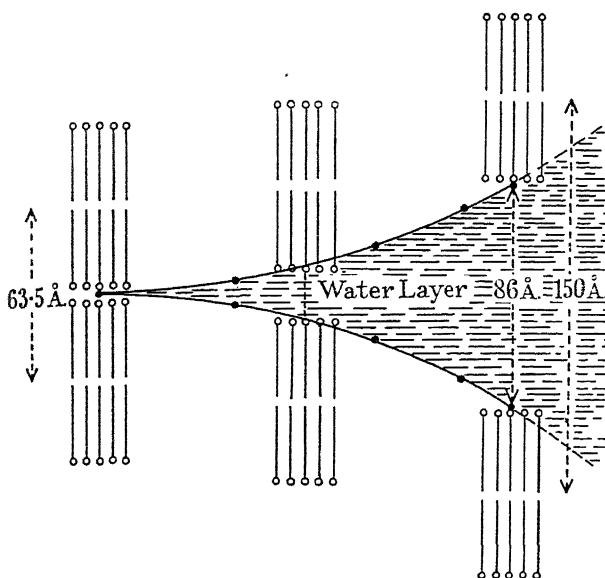


FIG. 23. Schematic representation of the thickness of the water layers between bimolecular leaflets of mixed lipoids as a function of the relative amount of water with which the lipoids were emulsified. Points represent values obtained when the water content was 0 (dry), 25, 50, 67 and 75%.

the absence of a high degree of selective permeability in the nerve sheath is associated with the presence of areas of large water content. For further discussion of this interesting branch of membrane study, especially in regard to the exact localisation of the protein and water constituents of the sheath and the effects of ions and narcotic substances on the fundamental structural pattern, the reader is referred to the papers of Schmitt (1936, 1939), Schmitt & Palmer (1940) and Schmitt & Bear (1939).

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CHAPTER VII

THE INTERPRETATION OF PERMEABILITY MEASUREMENTS

By J. F. DANIELLI

We have stated earlier that permeability studies are rarely made simply to obtain a list of constants for different substances, but rather to obtain information on the morphology and physiology of membranes. Before passing to their presentation we must accordingly consider to what extent we can interpret the experimental results so as to obtain this information. The paucity of definite correlations between permeability changes and cell or tissue function has also been emphasised earlier, so that the interpretations we are mainly concerned with in this chapter are those which aid in the elucidation of membrane structure.

Lipid Membranes. The work of Overton (1895-1900) introduced the first principle of membrane structural analysis. Overton found that substances penetrate cells in the same relative order as the order of the oil-water partition coefficients. Table V shows a typical example, taken from the experiments of Collander & Bårlund on *Chara ceratophylla*. Since this work of Overton it has been generally assumed that such a correlation indicates the presence of a large proportion of lipoid material in the plasma membrane and that substances with high oil-water partition coefficients penetrate rapidly because they are more soluble in the lipoid layer. In spite of occasional criticism, e.g. by Bayliss (1931), this hypothesis has held its ground and has now, more than forty years after its inception, been formally demonstrated Daniel

It was at one time thought possible that preferential permeability to lipoid soluble substances could be due to proteins, since most proteins have a considerable amount of fatty side-chains. But Collander (1927), investigating gelatin membranes, was unable to discover any preferential permeability to lipoid soluble substances. Many other proteins have more extensive amounts of lipoid side-chains than has gelatin, but in the view of the present authors this could at most explain such a degree

of preferential permeability to lipoids as is found with *Beggiatoa*, and even in this case we suspect that lipid molecules are probably also incorporated in the membrane.

TABLE V. SHOWING THE CORRELATION BETWEEN PERMEABILITY P AND THE OLIVE OIL:WATER PARTITION COEFFICIENT (B), FOR "LEAF" CELLS OF *Chara ceratophylla* (COLLANDER & BÄRLUND)

Substance	P	$B \times 10^2$
Methyl alcohol	0.99	0.78
Glycerol ethyl ether	0.077	0.74
Propylene glycol	0.087	0.57
Glycerol methyl ether	0.043	0.26
Ethylene glycol	0.043	0.049
Glycerol	0.00074	0.007
Erythritol	0.000046	0.003

In many cases it happens that permeability also runs parallel to surface activity, i.e. to the power of substances to accumulate at an air-water or an oil-water interface, and this led Traube (1904, etc.) to postulate an alternative mechanism to that of Overton, in which passage of substances was supposed to be facilitated by an initial increase in concentration, due to adsorption, at the cell surface. Partition coefficients run parallel to surface activity in the majority of cases, so that it was somewhat difficult to distinguish between the two hypotheses. This difficulty was further increased by the somewhat indiscriminate use of air-water, instead of the more appropriate oil-water interfaces, to measure surface activity. However, Traube's theory seems now to have fallen into general discredit, and his hypothesis is certainly not operative in many cases; consequently, in presenting the experimental evidence, Overton's hypothesis will be used throughout. There are, however, certain cases to which Traube's hypothesis is accurately applicable, as will be indicated in Chapter XXI.

Porous Membranes. Later it became evident that many membranes behave as though they were molecular sieves. A uniform molecular sieve will allow free passage of all molecules up to a certain diameter, and all molecules of greater diameter will be unable to penetrate. More generally, however—at least with artificial porous membranes—there appear to be a great many small pores but only a few large ones, so that penetration is restricted as molecular size is increased, without any sharp division appearing between penetrating and non-penetrating molecules. Only by taking very great care in preparation is it possible to obtain a

uniform pore size, as is the case with e.g. the membranes of Mane-gold & Bjerrum (1927), Elford (1930, 1933) and of Erbe (1933).

Systematic investigations of porous collodion membranes were made by Collander (1924, 1926), by Fujita (1926) and by Michaelis & Fujita (1925). Table VI shows some typical results, (*a*) for a very permeable membrane, and (*b*) for a less permeable membrane.

TABLE VI. VALUES OF $PM^{\frac{1}{2}}$ AND MR_D FOR TWO COLLODION MEMBRANES

Substance	(<i>b</i>)		MR_D
	very permeable	less permeable	
Methyl alcohol	6.9	5.2	8.2
Ethyl alcohol	7.8	2.0	12.8
Propyl alcohol	7.7	0.8	17.5
Butyl alcohol	7.3	0.7	22.2
Ethylene glycol	6.3	0.2	14.4
Glycerol	7.8	0.21	20.6
Glucose	7.3	0.0	37.5

For diffusion through large pores the laws for simple uncomplicated diffusion should apply; in this event the product $PM^{\frac{1}{2}}$ of the permeability constant and the square root of the mass of the penetrating molecules should be a constant (Thovert, 1910.; but for diffusion through narrow pores, having the same order of diameter as the diffusing molecules, $PM^{\frac{1}{2}}$ should fall off as the molecular diameter increases (Danielli). In Table VI values of the molecular refraction MR_D are given instead of molecular diameters. Molecular diameter varies approximately as $(MR_D)^{\frac{1}{3}}$, so that MR_D values are a good guide to molecular diameters, for moderately small molecules. It will be seen from the table that the $PM^{\frac{1}{2}}$ values do vary in this way, and that there is no evidence of preferential permeability by either the lipoid soluble series, methyl alcohol, ethyl alcohol, propyl alcohol, butyl alcohol, or for the water soluble series, methyl alcohol, ethylene glycol, glycerol, glucose, in the case of membrane (*a*). In the case of membrane *b*, $PM^{\frac{1}{2}}$ for glycol is only a quarter of the value for propyl alcohol, although propyl alcohol has a larger value of MR_D than has glycol. The same is true for butyl alcohol and glycerol. It is not clear whether this is due to propyl and butyl alcohols being adsorbed on the walls of the pores or dissolved in the substance of the membrane, or to the configuration of the monohydric alcohols giving them a diameter, across one axis, less than that of the polyhydric alcohols across all axes.

Calculation of $PM^{\frac{1}{2}}$ values* is thus a convenient measure for examining the nature of the pore factor in a simple porous membrane: if $PM^{\frac{1}{2}}$ is constant, the pore diameter must be much greater than that of the penetrating molecules; if at a certain molecular size $PM^{\frac{1}{2}}$ values fall practically to zero, the pore size must be that of the limiting molecular size: if $PM^{\frac{1}{2}}$ values fall to zero over a wide range of molecular size, the membranes probably contain pores of diverse sizes.†† For further discussion of these points, Weech & Michaelis (1928) and Höber (1936) should be consulted.

Porous Lipoid Membranes. Obviously such simple arguments will not suffice for a porous lipoid membrane, or for a homogeneous lipoid membrane superimposed upon a porous membrane. The former case should theoretically behave in the following manner: ethylene glycol, glycerol, erythritol, mannitol, monosaccharides, disaccharides should diffuse almost exclusively through the pores, i.e. in water, and should therefore obey the relationship $PM^{\frac{1}{2}} = \text{constant}$. If there are very few pores, glycol and glycerol may have values of $PM^{\frac{1}{2}}$ higher than is found for the other substances, due to a relatively significant amount diffusing through the lipoid areas. Thus for glycol, if we consider only the resistance to diffusion imposed by a membrane 100μ thick, the amount diffusing through the lipoid will be approximately equal to the amount going through the pores if the pores occupy only $1/10^9$ of the area of the membrane. For glycerol the two effects will only be equal when the pores occupy $1/10^{12}$ or less of the total area. In such cases the existence of the pores, if they are large, will always be revealed by the constancy of $PM^{\frac{1}{2}}$ for the large molecules. It is doubtful whether a very few *small* pores could be detected, since they would never significantly alter the permeability to any type of substance. Fatty substances will diffuse through the water-filled pores, obeying the relationship $PM^{\frac{1}{2}} = \text{constant}$, and in the interior of the lipoid material the relationship $PM^{\frac{1}{2}} = \text{constant}$ may possibly hold also. But entry into the lipoid layer will be roughly proportional to the oil-water partition coefficient, which varies enormously and has no relationship to $M^{\frac{1}{2}}$. Hence, if a

* Originally the ratio P/D , where D is the Fick diffusion constant, was used. If P/D is constant, the pore size must be large, etc. But also $DM^{\frac{1}{2}} = \text{constant}$, hence $PM^{\frac{1}{2}} = \text{constant}$. This is a more useful relationship, since M is much more often known than is D .††

large number of fatty substances were studied, we should find
 (a) a general tendency for P to fall as M increases; (b) a general
 tendency for P to rise as the oil-water partition coefficient increases.⁴⁴

However, these remarks have been based entirely on theoretical considerations, and insufficient work has been done upon the permeability of lipoid membranes of known structure for us to state confidently that these conclusions are correct.

Collander & Bärilund (1933), dealing with porosity superimposed upon a lipoid membrane, have suggested that P should be plotted against oil-water partition coefficient, and that if, on such a graph, a systematic relationship is revealed between P and molecular volume for each given value of the partition coefficient, such that the smaller the molecules the higher the permeability, then we may infer that the membrane is porous in some way. This has been further discussed by Collander (1937).

Mosaic Membranes. The suggestion was first advanced by Nathanson (1904) that cell-plasma membranes may be mosaics, consisting of areas of different properties. This concept differs from the pore concept in that, in the latter case, a discriminatory factor selects molecules according to their size, so that the discriminating units must be of the dimensions of molecular diameters. But according to the mosaic theory discrimination is exerted not according to molecular size, but according to the chemical and physical properties of penetrating molecules; in this case the discriminating units may be very large, and may consist of areas of many thousands of molecular diameters in extent. This suggestion has been more recently examined by Höber (1936). But conclusive proof of the existence of membranes of this type has yet to be given. There are a number of cases in which it is certain that the cell membrane is not homogeneous, but what form this inhomogeneity takes, whether it is a pore structure or a mosaic, can only be decided by more elaborate researches than have yet been undertaken.

Quantitative Tests. Recently, by applying the theory of activated diffusion to membrane problems, Danielli (Appendix A) has developed a number of quantitative tests for investigating the homogeneity of the cell membrane. These are: (a) If slowly penetrating molecules obey the relationship $PM^{\frac{1}{2}}Q_{10}^{(T+10)/10} = \text{constant}$, the membrane is homogeneous to a first approximation. The Q_{10} is that of the permeability. If this relationship is not obeyed, the

membrane cannot be homogeneous. Molecules used for this test must be chosen according to the method given by Danielli (Appendix A): the same molecular species cannot be used indiscriminately for examining all cells, since some molecules enter one type of cell slowly, and other types of cell rapidly. (b) If for any two molecules $PM^{\frac{1}{2}}$ for the molecule with the lower Q_{10} is not greater than $PM^{\frac{1}{2}}$ for the molecule with the higher Q_{10} , the membrane is not homogeneous. (c) If for slowly penetrating molecules $PM^{\frac{1}{2}} e^{\frac{2500x}{RT}}$ plotted against oil-water partition coefficients,* or for rapidly penetrating molecules $PM^{\frac{1}{2}}$ plotted against partition coefficient, gives a roughly linear relationship, the membrane is probably homogeneous to a first approximation. These relationships are a little difficult to use in practice, since oil-water partition coefficients vary greatly in value for different oils, so that it is not possible to predict the exact values which are true for the membrane of any particular cell.

These three tests, (a), (b) and (c), are strictly valid only for molecules which have an exterior surface almost entirely composed of polar groups, such as the polyhydric alcohols and sugars. To what extent the method is valid for molecules containing non-polar groups, such as the series of monohydric alcohols (methyl, ethyl, propyl, butyl, etc. alcohols), requires further experimental investigation.

Even these quantitative tests must be taken as provisional only, owing to our ignorance of some of the details of the mechanism of diffusion. Thus they are all dependent to some extent on the accuracy of equation (53) (Appendix A).

Electrical Measurements. The main assumption at the base of these measurements is that a change in the permeability of the membrane to ions will be reflected in a change in the electrical resistance and capacity of a cell or tissue. If a membrane is less permeable to any ionic species than is the surrounding medium, the cell will behave as a condenser in an electrical field; the efficiency of this cell as an electrical condenser will depend on the degree of impermeability of the various ions; consequently, if marked changes in the capacity of a cell suspension or tissue are observed when the environment is changed or when the tissue undergoes activity, the changes may be due to variation in the ionic

* x equals number of non-polar groups, such as CH_2 , per molecule.

permeability of the membrane. On the other hand, sometimes the resistance (i.e. permeability to ions) may change greatly without much change of capacity. A variety of such measurements are to be found in the literature, e.g. by Höber, Osterhout, Gordon

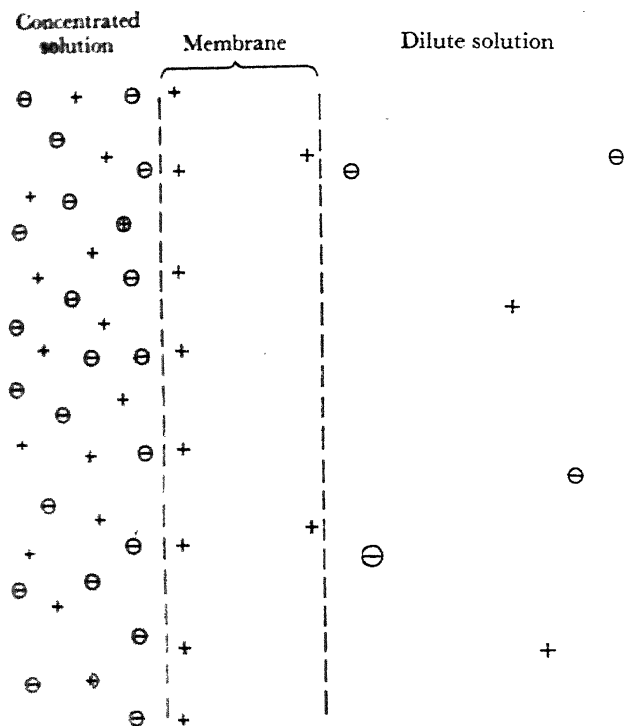


FIG. 24. Diagram illustrating the origin of a potential across a cation-permeable membrane between two salt solutions of unequal concentration.

& Hogben, McClendon, Lullies, Schmitt, Blinks, Hodgkin, Danielli, Fricke, Curtis, Cole, etc. (see Chapter xv). A more sensitive method is to measure the electrostatic potential difference across the cell membrane; interpretation is, however, complicated by the fact that the potentials observed are usually rather closely linked with cell metabolism.

Potential difference studies may often be used to decide whether membrane is selectively anion or cation permeable; the principle

used is based on the difference in sign of the potential found across the membrane when the two sides of the membrane are bathed in salt solutions of different concentrations. If the side with the higher concentration has a residual negative charge, then it follows that cations must be penetrating the membrane more rapidly than anions, the anions being "left behind" by the cations diffusing into the membrane from both the dilute and concentrated solutions, as shown in Fig. 24, but the stronger effect will be produced on the more concentrated side; hence the latter side will be more negative. *Mutatis mutandis*, if the membrane is preferentially permeable to anions, the more concentrated solution will be positive. This argument however is inexact, although it usually leads to a correct conclusion. Chapter xv should be consulted for a detailed consideration of potential measurements.

Use of Narcotics and Enzyme Poisons, etc. In many cases it is not clear whether the permeability observed is the result of a simple diffusion process, or of a secretory process. In the latter case the rate of penetration should be a function of metabolism. Whether this is so or not can be ascertained in some cases by either removing the normal metabolites used, such as oxygen and glucose, or by adding an enzyme poison such as cyanide, fluoride, etc. Such investigations have been made by Höber (1933), Huf (1936), Kekwick & Harvey (1934), Hunter (1936), Wilbrandt (1937), Davson & Danielli (1938) and Davson & Quilliam (1940), among others.

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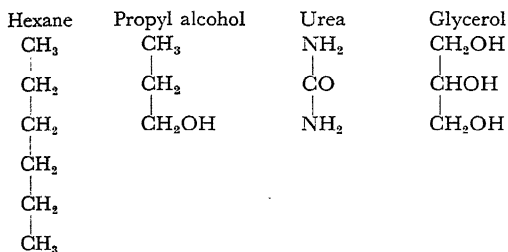
CHAPTER VIII

PERMEABILITY TO NON-ELECTROLYTES

By J. F. DANIELLI

By non-electrolytes we refer to substances which, over the physiological pH range, exist in a non-ionic form only and which also penetrate in an unionised form. Other substances (weak acids and weak bases) penetrate in most cases mainly in the unionised form, but they present several special problems which make it preferable to deal with them separately.

Within the group of non-electrolytes a wide diversity of chemical and physical characteristics is still possible. Consider the molecular series hexane, propyl alcohol, urea and glycerol. Hexane has an olive oil:water partition coefficient of about 500, whereas that of glycerol is 0.00007. Propyl alcohol and urea have intermediate values of about 0.1 and 0.00015. Thus there is a difference of ten million-fold between the relative "affinities" of



hexane and glycerol for water and oil. It is not surprising that such enormous differences between molecules should lead to great differences in permeability, provided the membrane to be penetrated can distinguish between molecules by virtue of these specific characteristics. Thus in water the dominant variable affecting the rate of diffusion on passing from one of the molecules to another is the molecular weight, so that the relative rates of penetration of a water layer are as 1.0 : 0.83 : 0.83 : 1.02; i.e. there is very little difference between the rates. In diffusing through e.g. a collodion membrane between two water layers, the

discriminating factor is molecular diameter, and again there would be very little difference between these four molecules. Fujita found with two different collodion membranes the following relative permeabilities for the last three of these substances: membrane (1) 1.0, 1.0, 0.8; membrane (2) 1.0, 0.97, 0.21. On the other hand, with cell membranes the ratios would be, roughly, hexane greater than 1.0 : propyl alcohol 1.0 : urea 0.005 : glycerol 0.0005.* The discrimination now is between molecules of different polar character, the least polar penetrating most rapidly, the most polar least rapidly. Bearing in mind these very large differences which may exist between different non-electrolyte molecules, we may now proceed to consider the permeability of a number of different types of membranes.

The Permeability of the Erythrocyte. The earliest systematic studies of the permeability of erythrocytes to non-electrolytes are those of Griens (1896) and of Hedin (1897), and it will be seen that, although their work was done so long ago, at a time when even the conception of the erythrocyte as a cell had first to be argued, their results are largely upheld by later work; in fact, it is worth pointing out that the work of Hedin was carried out under more strictly physiological conditions than most of the later investigations on non-electrolyte permeability.

Quantitative work on the erythrocyte is comparatively recent, and is almost entirely based on the haemolysis technique. The quantitative analysis of times of haemolysis is almost entirely due to Jacobs and his colleagues. For many points we are still dependent of qualitative evidence.

Griens first showed, by chemical methods, that urea added to chicken blood is equally distributed between cells and serum. He then, using the haemolysis method, showed that the following non-electrolytes did not penetrate the cell at all: amino acids, glucose, mannose, inositol, disaccharides. The following substances were shown to penetrate the cell: MeOH, EtOH, glycerol, ethers, urea, biuret, pyridine.

Griens was mainly interested in whether the substances penetrated the erythrocyte or not, and paid little attention to the actual rates at which this process occurred. However, he was able to draw the important conclusion that "substances with the same

* There are, of course, many cases where specific factors cause displacement in this series.

chemical groupings behave similarly", which with modifications relating to the size of the molecules, and the distribution of the groups in them, may be upheld to-day.

Hedin, using the method of freezing-point depression, and ox blood, obtained the results summarised in Table VII. He discusses his results in relation to Overton's theory of lipid solubility, the more rapidly penetrating substances being the more lipid soluble substances, with the exception of urea and ethylene glycol. He enunciates the rule that with polyhydric alcohols the rate of penetration decreases with the number of OH groups in the molecule. Thus already the principle of the importance of specific chemical groupings in regard to permeability had been brought out.

TABLE VII. PERMEABILITY OF OX ERYTHROCYTES
(HEDIN, 1897)

Cane sugar)	Do not penetrate
Dextrose	
Galactose	
Arabinose	Penetrate only slightly within 24 hours
Mannitol	
Adonitol	
Ethylene glycol)	Penetrate in the order given, times for completion of the process being a few minutes for ethylene glycol, less than 2 hours for glycerol and greater than 28 hours for erythritol
Glycerol	
Erythritol	
Amides	Penetrate too rapidly for rates to be compared
Urea	
Aldehydes	
Ketones	
Ethers	

Besides developing the above principle, the results of Kozawa (1914), using a haemolysis technique, led to the conception of real differences in permeability according to the species of erythrocyte studied. Thus with human erythrocytes he found the results shown in Table VIII. The erythrocytes of the ape (*Macacus rhesus*) behaved in the same way as human erythrocytes. It was

TABLE VIII. PERMEABILITY OF HUMAN ERYTHROCYTES
(KOZAWA, 1914)

Permeable to:	Arabinose, xylose, galactose, mannose, sorbose, glucose, laevulose
Slightly permeable to:	Adonitol
Impermeable to:	Cane sugar, maltose, lactose, glucoheptose, methylglucoside, galactoside, glycine, alanine, mannitol, dulcitol

found, however, that the erythrocytes of the ox, pig, rabbit, guinea-pig, goat, horse, camel and cat were all impermeable to the substances considered; dog cells were shown to be permeable to glucose. The important point was also made that isomeric sugars penetrate at different rates, and has since been confirmed by Wilbrandt (1938).

TABLE IX. TIMES OF HAEMOLYSIS IN ETHYLENE GLYCOL AND GLYCEROL SOLUTIONS FOR DIFFERENT SPECIES (JACOBS, 1931)

Species	Time in seconds for 75% haemolysis				
	(1)	(2)	(3)	(2)-(1)	3-1
	In NaCl	In glycol	In glycerol	(1)	1
Rat	4.20	6.6	19	0.5	3.5
Mouse	3.00	8.6	39	1.9	12.9
Rabbit	3.00	11.3	80	2.2	21.8
Guinea-pig	3.00	15.7	196	2.1	38.2
Man	8.35	12.6	43	0.5	5.1
Dog	6.16	28.6	1548	3.7	253.0
Cat	2.65	18.3	1222	5.9	459.0
Pig	3.00	16.7	1024	4.6	340.0
Ox	3.80	35.1	2325	8.3	612.0
Sheep	1.90	24.1	1623	11.7	859.0

Column (1) gives time of haemolysis in 0.02 M NaCl; column (2) that in ethylene glycol + 0.02 M NaCl; column (3) that in glycerol + 0.02 M NaCl

TABLE X. (ULRICH, 1934)

Species	Substance	Penetration
Ox, pig, rabbit, rat	Hexoses	Not at all
Mouse	Hexoses	Not at all
Mouse	Sorbitol, mannitol, dulcitol	Rapid
Guinea-pig	Pentoses, glycine, alanine	Penetrate

Since Kozawa's work appeared, a great many investigators have made more detailed studies of the variation in permeability with species. In Table IX some results of Jacobs on the relative rates of penetration of ethylene glycol and glycerol into the cells of ten species are shown. The results are given in times of haemolysis, which are, very roughly, inversely proportional to permeability. The polyhydric alcohols and sugars have been studied from a species point of view by Ulrich (1934), whose results are shown in Table X. For the polyhydric alcohol, erythritol, the times in Table XI were found for 75% haemolysis.*

* It will be seen later, when Davson's (1939) results on loss of potassium in non-electrolyte solutions are discussed, that times of haemolysis of more than a few minutes are sometimes of doubtful value.

PERMEABILITY TO NON-ELECTROLYTES

TABLE XI. HAEMOLYSIS IN ERYTHRITOL SOLUTIONS

Species	Time for 75 % haemolysis
Ox	8 hours
Rat	6 hours
Pig	5 hours
Rabbit	3.5 hours
Man	1.75 hours
Mouse	Few minutes

Jacobs & Glassman in a preliminary report (1937) give a brief account of results on species characteristics, the most important of which are summarised below. The permeabilities to glycerol, urea and thiourea of the erythrocytes of 9 species of elasmobranch, 14 of teleosts, 2 frogs, 6 turtles, 4 snakes and 4 birds were compared. The following generalisations may be made:

- FISHES. Ethylene glycol penetrates fastest. Urea and thiourea highly variable from species to species. Thiourea usually faster than urea.
- BIRDS. Glycol and glycerol penetrate very rapidly at nearly equal rates. Thiourea much slower, urea slowest.
- REPTILES. Urea relatively rapid, glycol next; thiourea much slower. Permeability to glycerol only slight.
- MAMMALS. Urea very rapid; ethylene glycol much slower and thiourea still slower.

The most interesting features of the work so far described on species characteristics are first, that species may be grouped in accordance with their permeability to a given substance, e.g. glycerol; second, that many species show a specially high permeability to a given substance which is out of all proportion to its permeability to other substances; e.g. human cells are permeable to glucose, whilst rat and mouse cells are not at all permeable to glucose, but are more permeable to glycerol than are human cells: further, mouse cells are permeable to the polyhydric alcohols, sorbitol, mannitol and dulcitol, whilst human cells are not.

So far enough has been said of species characteristics *per se* for the reader to appreciate how real and significant they are; in the later parts of this section further species characteristics will be brought out in relation to other phenomena of non-electrolyte penetration.

The question of the relation of the rate of penetration of a substance with a low lipid solubility to its molecular volume has been investigated by Mond & Hoffmann (1928) and Mond & Gertz (1929). More recently Höber & Ørskov (1933) have made a more extensive study from the same point of view, and, as their results agree with those of Mond & Hoffmann, it will be sufficient to quote them alone.

The method used was the haemolysis technique, and some of the results have been collected in Table XII. The figures in the table are values of $t' = (t_2 - t_1) \cdot t_1$, where t_1 = time of haemolysis in 0.02 M NaCl, and t_2 = time of haemolysis in non-electrolyte + 0.02 M NaCl. In this way differences in the fragility of the cells are to some extent discounted.

RELATIVE TIMES t' OF HAEMOLYSIS OF ERYTHROCYTES OF DIFFERENT NON-ELECTROLYTES (SEE TEXT FOR DEFINITION OF t') HÖBER & ØRSKOV, 1933

Species	Acetamide	Propionamide	Lactamide	Malonamide
M.R.	14.9	19.5	21	22.9
Rat	0.47	0.45	18.4	167
Man	0.90	1.20	31.5	1025
Ox	1.30	1.70	72.0	890
Species	Urea	Methyl-urea	Thiourea	
M.R.	13.7	18.5	19.6	
Rat	0.11	1.9	31.7	
Man	0.3	2.3	57	
Ox	0.4	3.0	119	
Species	MeOH	EtOH	PrOH	BuOH
M.R.	8.2	12.8	.	.
Rat	0	0.11	0.45	0.45
Man	0.125	0.3	0.3	0.7
Ox	-0.19	-0.05	0.03	0.41
Species	Glycol	Glycerol	Erythritol	
M.R.	14.4	20.6	26.8	
Rat	0.94	4.7	1,500	
Man	1.7	60	10,750	
Ox	16	650	Infinite	

& Ørskov conclude that the absolute value of the "Molecular Refraction", i.e. the volume of the molecule, is only the determining factor when a given series of homologous substances is considered. The basis for this is clear from the table; in each group of homologous or chemically similar substances the value of t' increases as the molecular volume increases. On the

other hand, for a given species, there is no correlation between the rates of haemolysis and the molecular volumes when substances belonging to different homologous series are used. The results of Höber & Örskov were extended to nine species and differences as striking as those described earlier were observed.

TABLE XIII. (JACOBS *et al.* 1935)

Species	Substance	Time of haemolysis in seconds
Ox	Glycerol	2169
	Triethylene glycol	31
	Diethylene glycol	58
	$\alpha\gamma$ Dioxyp propane	114
	Ethylene glycol	38
	$\alpha\beta$ Dioxyp propane	14
	Propyl alcohol	1
Rabbit	Glycerol	51
	Triethylene glycol	5
	Diethylene glycol	27
	$\alpha\gamma$ Dioxyp propane	82
	Ethylene glycol	17
	$\alpha\beta$ Dioxyp propane	7
	Propyl alcohol	1.4

The results of Höber & Örskov made it quite clear that besides the molecular volume of the molecule and its lipid solubility, there is at least one other factor of importance in determining the permeability of a membrane to a given substance. Hedin's finding that OH groups tend to slow the rate of penetration of a molecule has received ample confirmation in the study of the comparative rates of penetration of sugars and their analogous polyhydric alcohols (with the exception in this case of the mouse erythrocyte). Further evidence of the importance of specific chemical groupings is given by the work just quoted of Höber & Örskov, and Fleischmann (1928) has shown that although glucose penetrates rapidly into the human erythrocyte, methyl glucoside does not penetrate at all, thus emphasising the importance of the groups in the sugar molecule. Quantitative work on the effect of introducing OH groups into the propane molecule has been published by Jacobs *et al.* (1935) and is shown in Table XIII, and it is seen that on introducing successive OH groups into this molecule the rate of penetration decreased with each group introduced. In the dihydroxy compound it is evident that the position of the OH group is important, the compound with its OH groups on adjacent ($\alpha\beta$) carbon atoms penetrating

more rapidly than that with the OH groups at the ends of the molecule ($\alpha\gamma$).

Before proceeding with the remaining points of importance in relation to non-electrolyte permeability it would be useful to recapitulate the principles derivable from the work so far described, as follows: apparently important variables are

- (a) Species characteristics,
- (b) Lipoid solubility,
- (c) Molecular volume,
- (d) Specific chemical groupings,
- (e) Position of the groups on the molecule.

All of these factors, with one possible exception, are of fundamental importance in determining the rate of penetration of a substance. The exception is molecular volume. The results we have given above show without doubt that, in any one homologous series, as molecular volume increases rate of penetration decreases. But many other properties of an homologous series vary in the same way as molecular volume, so that whilst it is of interest to observe this general parallelism between rate of penetration and molecular volume, a quantitative treatment is necessary before we can be sure whether this correlation is fortuitous or not.

Conclusions on the Structure of Red Cell Membranes. Many results are available to show that if a series of cells, such as (a) rat, (b) human, (c) ox erythrocytes, is considered, substances are found to penetrate the cells in different orders. For example, Höber & Ørskov (1933) found that with acetamide the order of speeds is rat > man > ox, whereas with propyl alcohol the order is ox > man > rat. According to Jacobs *et al.* (1935) glycerol penetrates in the order rabbit > guinea-pig > rat > man > cat > ox; but with thiourea the order is cat > rat > rabbit > man > guinea-pig > ox. According to some authors the differences between such series are such that they can only be accounted for by a mosaic structure of the plasma membrane of at least some species; i.e. the membranes of these cells must consist of areas of different chemical properties. To the present writers this view does not seem to be well founded, since it is based on qualitative rather than on quantitative arguments.

In Table XIV are given values of the permeability, temperature coefficient and of $PM^{\frac{1}{2}}Q_{10}^{T-10:10}$ for ox red cells. The experimental data were taken from Jacobs *et al.* (1935) and the values of P calculated by the method of Jacobs (1934). For the substances

glycerol, $\alpha\gamma$ dioxyp propane, diethylene glycol, triethylene glycol, the value of $PM^{\frac{1}{2}} Q_{10}^{(T-10)/10}$ should be approximately constant, but not for the other molecules, if the membrane is a homogeneous lipid layer.* It will be seen that this is the case, so that the membrane of ox red cells behaves, to a first approximation, as a homogeneous lipid layer.††

TABLE XIV. DATA FOR OX RED CELLS AT 20° C.

Substance		Q_{10}	
Propyl alcohol	10.6×10^{-16}	1.37	1.1×10^{-10}
Urea	7.8×10^{-16}	1.86	8.5×10^{-7}
Thiourea	0.019×10^{-16}	2.14	1.7×10^{-7}
Glycol	0.209×10^{-16}	2.92	2.0×10^{-2}
$\alpha\beta$ Dioxyp propane	0.405×10^{-16}	3.75	82
Glycerol	0.001×10^{-16}	3.65	0.17
$\alpha\gamma$ Dioxyp propane	0.105×10^{-16}	3.31	0.48
Diethylene glycol	0.075×10^{-16}	3.42	1.16
Triethylene glycol	0.033×10^{-16}	3.34	0.28

On the other hand, in the case of red cells of man, rat and rabbit, it can be shown that a small part of the surface of the cell is specially adapted to allow passage of glycerol. Results of Jacobs *et al.* (1935) show that for these cells the Q_{10} is about 1.2 for glycerol, and about 1.4 for urea. Consequently, if the membrane were homogeneous, glycerol should penetrate these cells more rapidly than urea.* But in fact urea penetrates thirty-five times or more faster than glycerol. This can only be accounted for if 3% or less of the surface of these cells is specially differentiated to permit passage of glycerol at a high rate.††

The Influence of the Medium on Permeability of Cells to Non-Electrolytes. So far as we are aware, no extensive systematic study of this aspect of non-electrolyte permeability has been described and most of what is known arises out of accidental discoveries. The principal work in this field is that of Jacobs and of Davson.

Jacobs *et al.* (1935) mentioned that "acidity" of the medium causes, with a group of species, a marked retardation of the rate of haemolysis in glycerol solutions; the effect seems to be confined to this molecule. These authors do not define the pH at which the effect of acidity shows itself. In the description of the equilibrium conditions of the erythrocyte in Chapter III it was

* See Chapter VII and Appendix A for the evidence and qualifications attending these statements.

shown that the non-electrolyte suspension medium is acid and may have a pH of 5 or even less, so that the effect of acidity described by Jacobs *et al.* must show itself at even more acid reactions. Davson (1939*d*) has investigated the matter further and some representative results on the rabbit erythrocyte, using a phosphate buffer, are shown in Table XV, and it seems clear that the retarding influence of acidity begins at a pH of from 4.7 to 4.5; however, in view of the importance of the ionic strength of the medium in these almost completely electrolyte-free suspensions of erythrocytes, it is questionable whether the pH of the medium alone is of much significance. The fact that the effect of acidity may be revealed by the "incautious breathing" of the investigator gives some idea of the difficulties encountered in a great deal of the work on penetration of non-electrolytes.

TABLE XV. THE EFFECT OF THE pH OF THE MEDIUM ON THE RATE OF HAEMOLYSIS OF RABBIT RED CELLS IN ISOTONIC GLYCEROL SOLUTION.

Suspension medium	Time of haemolysis
0.32 Glycerol	30 sec.
0.32 Glycerol buffer, pH 7.4	48 sec.
0.32 Glycerol buffer, pH 6.8	41 sec.
0.32 Glycerol buffer, pH 5.9	39 sec.
0.32 Glycerol buffer, pH 5.5	34 sec.
0.32 Glycerol buffer, pH 5.0	30 sec.
0.32 Glycerol buffer, pH 4.75	53 sec.
0.32 Glycerol buffer, pH 4.5	320 sec.

influence of the medium on permeability is described by Jacobs & Corson (1934) in a short note. These authors state that minute traces of copper in the solution of glycerol causes a pronounced retardation in the rate of haemolysis of certain species of erythrocytes, attributable presumably to a slowing of the rate of entry of glycerol. The effect shown is with those species which show the effect of acidity: man, rat, mouse, guinea-pig, rabbit. It is inhibited by $NaHCO_3$.

Davson (1939*d*), in a general study of the action of heavy metals, has shown that the effect of copper can be greatly increased by washing the cells with saline, this action being due to the washing out of the bicarbonate and removal of the serum proteins from the system. Addition of $NaCl$ or any alkaline buffer inhibits the action of copper, the pH at which this action begins being as acid: These facts are shown in Tables XVI and XVII.

Jacobs had noticed that the time course of haemolysis in glycerol in the presence of copper is peculiar in that there is a marked auto-acceleration towards the end of the process. Davson has found an explanation for this, attributing this effect to increase in alkalinity of the suspension medium, due to the haemolysis itself. As alkalinity inhibits the action of copper, it will therefore accelerate the rate of penetration of glycerol. The alkalinity following haemolysis is due to the breakdown of the equilibrium distribution of anions, which for a cell in a non-electrolyte medium will be such as to cause the cell to be alkaline in respect to the suspension medium. Probably adsorption of copper on the haemoglobin released by haemolysis is also an important factor in accelerating haemolysis.

TABLE XVI. EFFECT OF WASHING OF ERYTHROCYTES ON THE INHIBITION OF PENETRATION OF GLYCEROL BY COPPER (DAVSON,

	Suspension medium	Time of haemolysis
Unwashed cells	0.32 <i>M</i> Glycerol	0 min. 35 sec.
Unwashed cells	0.32 <i>M</i> Glycerol 1×10^{-5} <i>M</i> Cu	2 min. 55 sec.
Once washed cells	0.32 <i>M</i> Glycerol 1×10^{-5} <i>M</i> Cu	6 min. 30 sec.
Twice washed cells	0.32 <i>M</i> Glycerol 1×10^{-5} <i>M</i> Cu	7 min. 25 sec.

TABLE XVII. INFLUENCE OF REACTION OF THE MEDIUM ON THE INHIBITING ACTION OF COPPER ON GLYCEROL PENETRATION, AS SHOWN BY CHANGES IN TIME OF HAEMOLYSIS (IN SECONDS) (DAVSON,

The time of haemolysis in glycerol alone was 30 sec.

pH	Glycerol + buffer	Glycerol + buffer + 10^{-5} <i>M</i> ^
6.75	41	44
4.75	52	53
4.5	320	600

Jacobs *et al.* (1937) have investigated the effect of small quantities of salts on the rate of haemolysis of ox cells in glycerol solutions and find a marked acceleration, which, however, they attribute chiefly to a change in the fragility of the cells due to anionic shifts. It is possible that other factors may be involved.

A possible influence of salts and plasma proteins on penetration is revealed by Somogyi's (1928) results on the distribution of glucose between human plasma and cells; Somogyi's claims, that the passage of glucose from the cells to the suspension medium takes place within a minute or two, would give a permeability

constant of the same order as that of glycerol, and yet erythrocytes of man are stable in pure glucose solutions for hours at room temperature. As most of the work on non-electrolyte permeability has been done with the haemolysis technique, which involves an enormous reduction of the salt content of the medium surrounding the cell, it seems that the thorough investigation of the influence of environment on permeability to non-electrolytes would not only be interesting, but absolutely necessary, before the exact significance of results obtained by the haemolysis technique will be clear.

In concluding this description, it should be pointed out that we have not attempted to include all the published work on non-electrolyte permeability in this brief account, so that a certain amount of selection has been necessary; thus the voluminous literature on the vexed question of the distribution of glucose and amino acids between cells and serum has not been touched upon.

Plant Cells. A very large amount of work has been done on the permeability of plant cells including yeasts, but of this extremely little is published in a form suitable for quantitative treatment. The most outstanding work is that of the Finnish school of Collander & Bärlund. Fig. 25 shows the permeability of cells of *Chara corallina* plotted against oil-water partition coefficient—a form of graph originally due to Collander & Bärlund 1933. The larger the oil-water partition coefficient, and the smaller the molecular volume, the more rapidly a molecule penetrates. This work is based entirely on chemical determination of the amount of penetrating substance which actually enters the cell sap, a fact which makes the work most reliable. From this diagram Collander 1937 concludes that "There is clearly a somewhat close concordance between the oil-water solubility of substances on the one hand, and their permeability constants on the other; this is not merely a general concordance, but, at least approximately, a direct proportionality.... On the other hand, the smallest molecules obviously permeate faster than would be expected on account of their oil solubility alone.... It seems therefore natural to conclude that the plasma membranes of the *Chara* cells contain lipoids, the solvent power of which is on the whole similar to that of olive oil. But, while the medium-sized and large molecules penetrate the plasma membrane only when dissolved in the lipoids, the smallest molecules can also penetrate in some other

way. Thus, the plasma membrane seems to act both as a selective solvent and as a molecular sieve."

This view of Collander's is based on qualitative arguments only. (Appendix A), from a theoretical study of the details of

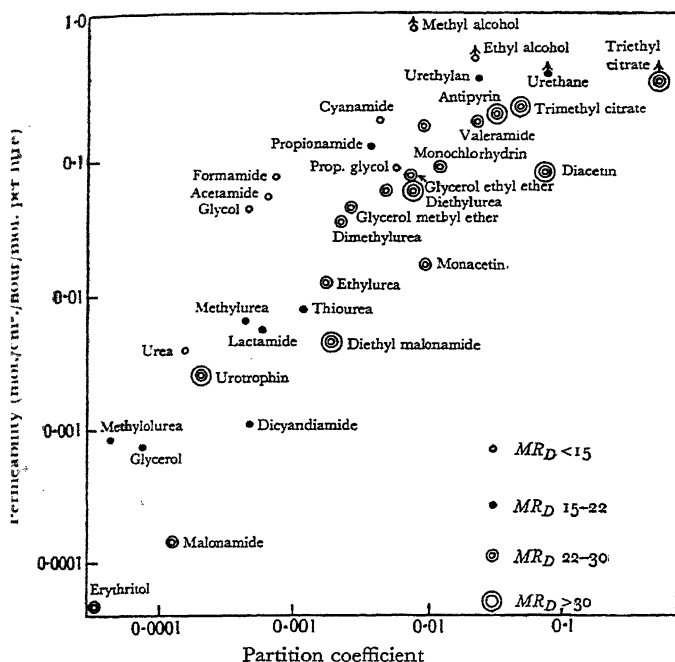


FIG. 25. Permeability (ordinates) of *Chara* to non-electrolytes (cm. per hr.) plotted against olive oil-water partition coefficients (Collander, 1937). Arrows are attached to permeability values which Collander & Bärlund consider to be too low.

the mechanism of diffusion across the plasma membrane, concludes that there is no justification for plotting P against partition coefficients, so that the conclusions which Collander draws from Fig. 25 are not valid. Instead, one should plot* $PM^{\frac{1}{2}}$ (in the case of rapidly penetrating molecules), and $PM^{\frac{1}{2}}e^{\frac{2500x}{RT}}$ (in the case of slowly penetrating molecules), against partition coefficients. Then;

* See p. 80 or p. 352 for explanation of these formulae.

for a homogeneous lipid layer, the points should lie between two lines distant by a factor of 5 from the average line. As can be seen from Figs. 26 and 27 the results of Collander & Bärlund fall within these limits, and there is no differentiation between molecules of different molecular volume. Hence it is concluded that the plasma membrane of *Chara* cells is possibly a homogeneous lipid layer, to a first approximation. Thus, within the range of molecular species studied, molecular volume is not a variable affecting the rate of penetration, and the reason why Collander & Bärlund found a degree of correlation between molecular volume and rate of penetration is that molecular volume tends to run parallel to the quantities $M^{\frac{1}{2}}$ and $M^{\frac{1}{2}} e^{\frac{2500\mu}{RT}}$, so that there is also a fortuitous correlation between P and molecular volume.

Collander also gives data for fifteen other plant cells studied in the Helsingfors laboratory, shown on Fig. 28. The cells are arranged in order of increasing permeability to erythritol. Cells of flowering plants, mosses, green algae, diatoms, brown algae, red algae, blue-green algae and bacteria are represented. Collander (1937) interprets all these results in terms of the "lipoid-sieve" theory. He remarks "1. *Spirogyra* and *Chara* are seen from Fig. 28 to agree very closely as to their permeability, except that the permeability of *Chara* cells is about three to ten times greater than that of *Spirogyra* cells. Such a difference can, at least theoretically, be explained on the assumption that the membrane of *Spirogyra* is correspondingly thicker than that of *Chara*. (2) The epidermal cells of *Rhoeo* have an exceptionally low permeability to all amides. . . This can easily be explained along lines first put forth by Hober and his school* . . . we have only to assume that the plasma membrane lipoids of most cells are acidic in character, while those of *Rhoeo* are approximately neutral. (3) The root cells of *Lemna* differ from most other plant cells in being more permeable to urea than to the more lipid soluble methyl urea. Perhaps this can be explained on the assumption that the plasma membrane of the cells in question contains a considerable number of pores of such a diameter as to be just penetrable by the urea molecules but not by the somewhat greater molecules of methyl urea. (4) The two diatoms so far studied, viz. *Melosira* and *Licmophora*, are both characterised by their remarkably high permeability to

* Hober (1930), Wilbrandt (1931).

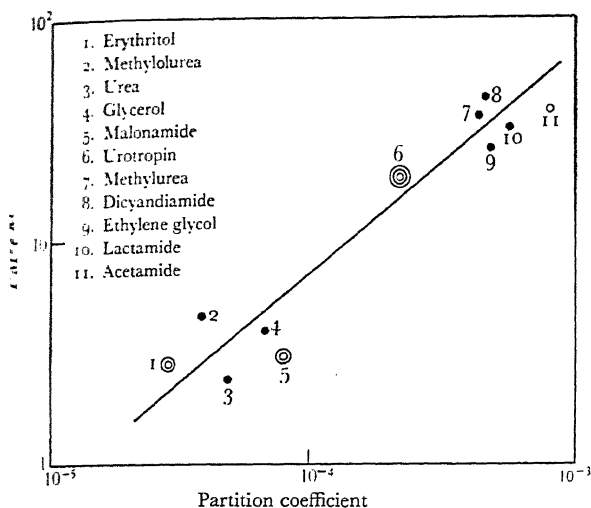


FIG. 26. $PM^{1/2}$ for non-electrolytes penetrating *Chara*, plotted against olive oil-water partition coefficients. The points are not more distant than a factor of 5 from the straight line, showing the membrane to be homogeneous to a first approximation.

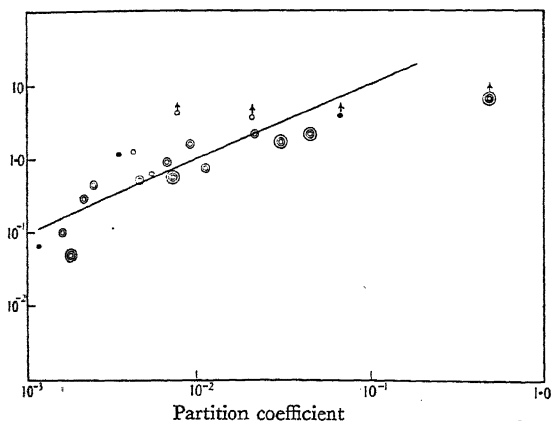


FIG. 27. PM^1 for non-electrolytes penetrating *Chara*, plotted against olive oil-water partition coefficients. The points are not more distant than a factor of 5 from the straight line, showing that the membrane is homogeneous to a first approximation. Arrows are attached to permeability values which Collander & Bärilund consider to be too low.

erythritol and sucrose, i.e. to substances which have an extremely low lipid solubility and a considerable molecular volume. This points to the occurrence of plasma membrane pores of an extreme width in these cells. 5 A great abundance of somewhat smaller plasma membrane pores may be assumed in the case of *Oscillatoria*.

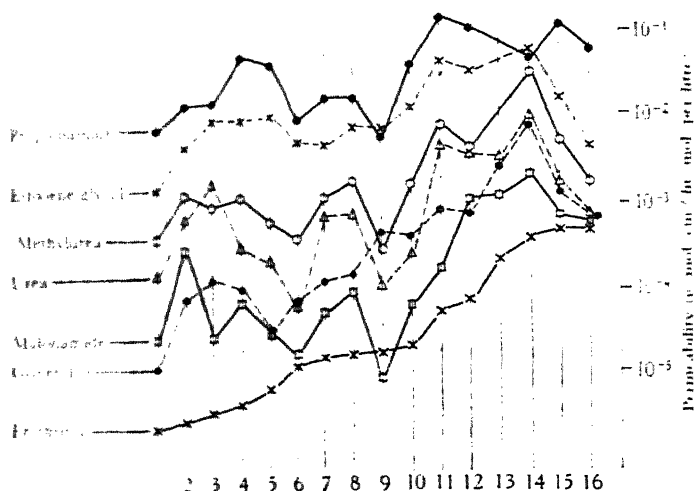


FIG. 28. Permeability (cm. per hr.) of plant cells to non-electrolytes: 1 leaf cells of *Pinguicula denticulatum*, 2) *Oedogonium* sp., 3) root cells of *Lenaxa minor*, 4) *Pisum sativum*, 5) *Synema cyanosporum*, 6) subepidermal cells of *Cannabis sativa*, 7) *Spirogyra* sp., 8) leaf cells of *Elodea densa*, 9) epidermal cells of *Rhoeo dasycarpa*, 10) epidermal cells of *Taraxacum pectinatifolium*, 11) "leaf" cells of *Chara coralliphylloides*, 12) internodal cells of *Ceratophyllum demersum*, 13) *Baccharis peruviana*, 14) *Oscillatoria princeps*, 15) *Melosira* sp., 16) *Lichophora* sp. after Gollander, 1937.

which differs from most other cells in that the sieve principle is more dominant and the effect of the lipid solubility less so than in the other cases."

However, as was the case with *Chara*, these arguments are entirely qualitative, and unfortunately the results available for these various cells are hardly sufficient for any positive conclusion to be obtained from quantitative examination. It may, however, be said that with one possible exception, if the same quantitative tests are applied to the results given in Fig. 28 as to those for

Chara, e.g. if $PM^{\frac{1}{2}}$ is plotted against partition coefficient, the points for all these cells fall within the limits to be expected for a homogeneous lipid membrane, and that there is no particular evidence of any pore size factor being involved. See, for example, the results for *Melosira* plotted on Fig. 29. For all the studied plant cells the slope of the average straight line, passing through the values of $PM^{\frac{1}{2}}e^{\frac{2500x}{RT}}$ for slowly penetrating molecules plotted against partition coefficient, is greater than the slope of $PM^{\frac{1}{2}}$ for rapidly penetrating molecules, plotted against the partition coefficient, as is predicted theoretically (Danielli, Appendix A).

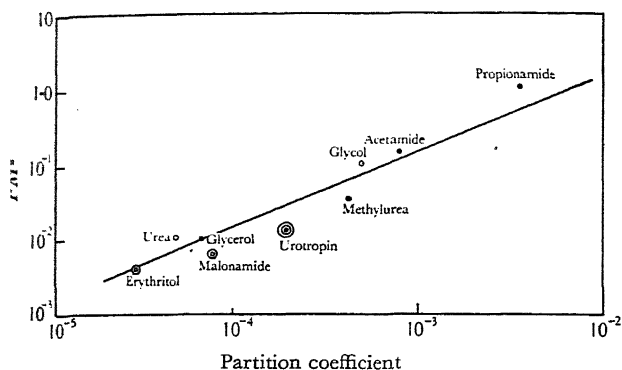


FIG. 29. $PM^{\frac{1}{2}}$ for non-electrolytes penetrating cells of the diatom *Melosira*, plotted against olive oil-water partition coefficients.

Permeability of Bacteria. Collander (1937) quotes a few values of P for *Bacterium paracoli* which are not greatly dissimilar from those of cells known to have a definite lipid membrane (see Table XXII).

A very large amount of work has been done on the sulphur bacterium *Beggiatoa mirabilis*, particularly by Ruhland & Hoffmann (1925) and by Schönfelder (1931). The work of the first two authors was largely responsible for the growth of interest in the view that the cell membrane behaves as a molecular sieve. Most of the published results are qualitative, and therefore do not permit of any final conclusion being made. Marklund (1936) gives a few values of P , which are quoted in Table XVIII, together with values of $PM^{\frac{1}{2}}$. The $PM^{\frac{1}{2}}$ values are constant within

experimental error with the exception of the value for saccharose. Consequently it may be said that the properties of the *Beggiatoa* membrane are those of a non-lipoidal molecular sieve, diffusion taking place through water-filled pores, many of which are of about the same diameter as saccharose molecules. There is no evidence of any correlation with partition coefficients. When the values of P are compared with those for other cells (Table XXII), it will be seen, however, that *Beggiatoa* has a permeability of the same order of magnitude as other cells, and as a layer of 50 Å.

TABLE XVIII. VALUES OF $PM^{\frac{1}{2}}$ FOR *Beggiatoa mirabilis*

Substance	P	$PM^{\frac{1}{2}}$
Saccharose	0.135×10^{-16}	2.5×10^{-16}
Erythritol	0.838×10^{-16}	9.3×10^{-16}
Glycerol	1.060×10^{-16}	10.2×10^{-16}
Urea	1.580×10^{-16}	12.2×10^{-16}
Methylurea	1.170×10^{-16}	10.1×10^{-16}
Glycol	1.390×10^{-16}	11.0×10^{-16}

of olive oil, but which is 10^9 times less than that of a water layer of the same thickness. Consequently, if the membrane is a non-lipoidal molecular sieve of the same thickness as other cell membranes, with penetration occurring through pores only, these pores must occupy an extremely small fraction of the membrane area—about one part in 10^9 . Now the total surface area of a single cell of *Beggiatoa* is about 5×10^{-5} sq. cm. Hence the total cross-sectional area of the pores is $5 \times 10^{-5} \div 10^9 = 5 \times 10^{-14}$ cm.² or 500 Å.² A pore large enough to admit saccharose has an area of about 30–40 Å.²; hence there can only be about 10–20 pores in the membrane of a *Beggiatoa* cell. If, therefore, we accept the molecular sieve hypothesis, either the greater part of the membrane of *Beggiatoa* must have a permeability equal to or less than that of plant and animal cells, with a very small number of wide water-filled pores occupying one part in a thousand million of the total membrane area; or else the membrane has more pores but is proportionately thicker.††

This seems a little peculiar, and it is perhaps worth pointing out that if we abandon the idea that the penetration of these cells is necessarily a simple diffusion process only, at least two possibilities are revealed which could explain their permeability: (1) pores having a diameter usually rather less than that of

saccharose appear periodically in the membrane, which is otherwise highly impermeable; (2) that *Beggiatoa* has two membranes, an outer membrane with an average pore diameter rather less than that of saccharose and an inner highly impermeable membrane, in which from time to time such a structure as a contractile vacuole opens, sucking material through the outer porous membrane.†† Thus much further work is needed before any final conclusion can be reached on the structure of the membrane of *Beggiatoa*.

A further alternative explanation of the behaviour of these bacteria is that the resistance to penetration due to the membrane is not so large that the resistance to diffusion through the cell contents may be ignored. In this case we are not justified in calculating the number of pores by the simple procedure given on page 102.

A similar calculation for *Bacterium paracoli* is still more interesting. Its membrane has an area of 3.4×10^{-8} cm. and a permeability of about 10^{-11} of that of a similar water layer, to molecules such as urea. If molecules were to penetrate through water-filled pores, the area occupied by the pores must be $3.4 \times 10^{-8} \times 10^{-11}$ cm.² = 0.0034 \AA^2 ; i.e. an area less than a molecular area, so small that even if the whole of this area were in one part it would be too small for any molecule to penetrate. Its membrane consequently cannot be an inert molecular sieve, unless it is enormously thicker than most plasma membranes.††

TABLE XIX. SHOWING THE FRACTION OF THE TOTAL VOLUME OF A BODY TAKEN UP BY A PLASMA MEMBRANE OF NORMAL STRUCTURE, IF PRESENT

Cell or virus	Smallest diameter	% of volume due to plasma membrane
<i>Beggiatoa mirabilis</i>	8 μ	0.1
<i>B. prodigiosus</i>	750 m μ	4
<i>B. coli</i>	250 m μ	3
Sewage micro-organism	150 m μ	19
Newcastle disease	100 m μ	27
St Louis encephalitis	25 m μ	75
Foot and mouth disease	10 m μ	100

Quite apart from experimental work, considerations of the space available inside a really small cell show that there is hardly sufficient space for a plasma membrane of the type found with large cells. Table XIX shows the percentage of "cell" volume taken up by a plasma membrane 50 \AA . thick in the case of

"cells" of various volumes. It will be seen that as soon as a size is reached at which a plasma membrane of normal thickness becomes a large part of the cell—in fact an expensive luxury—cells are no longer found, but only virus particles.^{4,5}

Marine Eggs. The first intentional study of the permeability of marine eggs is that of R. S. Lillie (1916, 1917, 1918), but his work was mainly confined to permeability to water. Work on non-electrolyte solutes is more recent, and is mainly due to Jacobs, Lucké & McCutcheon and their colleagues. Table XX gives results, mainly taken from Jacobs & Stewart (1936) on unfertilised *Arbacia* eggs. Q_{10} values are available for butyramide, propionamide and ethylene glycol, and for these three substances the value of $PM/Q_{10}^{T-10/10}$ is constant within experimental error. It follows that the *Arbacia* egg membrane is possibly homogeneous, but too few results are available to be certain of this. It will be seen from the table that the more lipoid-soluble substances tend to penetrate the faster, and that the more polar molecules penetrate more slowly.

TABLE XX. PERMEABILITY OF *Arbacia* EGG TO NON-ELECTROLYTES

Substance	P	Q_{10}	$PM/Q_{10}^{T-10/10}$
Butyramide	6.6×10^{-10}	3.8	2.65×10^6
Propionamide	2.3×10^{-10}	3.8	1.05×10^6
Acetamide	1.0×10^{-10}	.	.
Propylene glycol	1.3×10^{-10}	.	.
Ethylene glycol	0.73×10^{-10}	4.1	2.75×10^6
25 Dioxyp propane	0.67×10^{-10}	.	.
Diethylene glycol	0.43×10^{-10}	.	.
Glycerol	0.003×10^{-10}	.	.

Lucké *et al.* (1939) have compared *Chaetopterus* and *Cumingia* eggs with *Arbacia* eggs, finding that the former cells are considerably more permeable to glycol and glycerol than are *Arbacia* eggs. The permeability of *Arbacia* eggs to ethylene glycol and *xy* dioxyp propane is approximately doubled on fertilisation, as is also the case with water. But whereas the Na^+ , K^+ and Ca^{++} concentrations have a very marked effect on the permeability to water, the electrolyte content of the medium has little effect on permeability to glycol. From this Jacobs & Stewart (1936) conclude that the effect of electrolytes on the membrane is of a different nature from that of fertilisation.

Permeability of the Intestine, etc. There are many tissues which to some substances show a selective activity based on an active mechanism, but which to other substances, even of the same chemical group, are quite inert. Studies on the intestine furnish an interesting example of the methods which are adopted to distinguish between the two groups of substances. Two main methods have been used: (1) the effect of concentration of solute on the rate of absorption, (2) the effect of specific poisons on the rate of absorption.

TABLE XXI. THE EFFECT OF INITIAL CONCENTRATION ON THE AMOUNT OF SUBSTANCE ABSORBED FROM THE INTESTINES

Initial molarity	<u>Millimoles absorbed</u> <u>Initial concentration</u>
Urea (after 15 min.)	
0.06	0.83
0.09	0.76
0.12	0.69
0.18	0.80
0.24	0.71
Erythritol (after 15 min.)	
0.06	2.1
0.09	2.6
0.12	2.5
0.18	2.2
0.24	2.2
Valine (after 20 min.)	
0.056	2.5
0.084	1.5
0.115	1.1
0.167	0.6
0.223	0.4

Table XXI shows some results obtained by Höber & Höber (1937) on the effect of concentration on the amount of a substance absorbed by rat intestine. Urea and erythritol have values of $\frac{\text{Amount absorbed}}{\text{Initial concentration}}$ which, within experimental error, do not vary when the initial concentration is varied. This shows that for these substances the amount absorbed is directly proportional to the concentration in the intestine. But for valine the ratio falls off rapidly as concentration is increased, to an extent which suggests that, above a threshold, the amount of valine absorbed is independent of the concentration in the intestine. Höber

concludes that in general the polyhydric alcohols and the acid amides are absorbed at a rate proportional to their concentration and therefore probably penetrate by simple diffusion, but that with amino acids absorption is "complicated by the presence of an accelerating factor, the effect of which becomes more visible with lower than with higher concentrations". In the case of polyhydric alcohols absorption resembles diffusion through a sieve-like membrane, the maximum pore diameter of which is about that of mannitol, so that mannitol (C_6) is scarcely absorbed at all, but adonitol (C_5) and erythritol (C_4) are comparatively rapidly absorbed. But with the amides the effect of lipid solubility is of marked importance. Thus butyramide, with a molecular volume of 113, should penetrate a sieve more slowly than acetamide (molecular volume 69), but in fact butyramide penetrates rather more rapidly than acetamide.

The use of poisons is well illustrated by the work of Wilbrandt & Laszt (1933) on the absorption of sugars. These workers measured the rate of absorption of sugars (a) without iodoacetate, b with iodoacetate, and found, in a typical case, that the ratios of $a : b$ were: galactose 2.1, glucose 3.0, fructose 1.2, mannose 1.3, xylose 1.0, arabinose 1.0. It was concluded from this that an active mechanism, possibly phosphorylation, was involved in the transport of at least the sugars galactose and glucose. This theory will, of course, require much further experimental examination before it can be regarded as correct.

The intestine is such a complex tissue, and so complicated by active processes, that it does not seem profitable to make further comment upon it at the present time.

The Capillary Membrane. Despite its importance, extremely little is known about the permeability of the capillary membrane; it is probable that it will be found to resemble the glomerular membrane in being a molecular sieve of rather large pore size. According to Keys (1937) the available evidence shows that substances penetrate the capillary membrane in the order $H_2O > \text{urea} > \text{glucose} > \text{sucrose}$.

Chitin Membranes. An investigation of lobster chitin membranes by C. M. Yonge (1936) shows that the membrane is much more permeable to weak acids than to strong acids, and that for the weak acids the relationship, $PM^{\frac{1}{2}} = \text{a constant}$, is obeyed within experimental error (Table XXI a).

TABLE XXIIa. PERMEABILITY OF CHITIN

	M	P	$PM^{\frac{1}{2}}$
Hydrochloric acid	36	2.5	15
Formic acid	45	19	127.5
Acetic acid	60	13.5	104.5
Propionic acid	75	14	122
Butyric acid	89	13	123

There is no tendency for the rate of permeation to follow the oil-water partition coefficients of the molecules. The adherence to the relationship $PM^{\frac{1}{2}} = \text{constant}$, together with the relative impermeability to HCl, shows that the weak acids probably penetrate mainly in the undissociated form, that the penetration takes place through water-filled pores which are considerably greater in diameter than is butyric acid, and that the pore walls probably carry an electrical charge sufficient greatly to reduce the rate of penetration of ionic substances.

Relative Permeability of Various Cells. Table XXII gives values of the permeability P of fifteen different cells to non-electrolytes, compared with the "permeability" of layers of water and olive oil of about the same thickness as the plasma membrane. The water layer is always much more permeable than the cell membrane in the cases cited. This, however, does not mean that there are not some substances which may diffuse just as rapidly as through water. Such may be the case with hydrocarbons. But accurate permeability constants can at present only be determined for substances which do diffuse much more slowly through the cell membrane. What is of much more importance is that whereas in water all the substances concerned diffuse at about the same speed, through the cell membrane the rates differ by a factor of 10^6 , and that to the most rapidly penetrating of these molecules the membrane permeability is still 10^8 times less than that of a water layer of equivalent thickness.

The values given for the permeability of an olive-oil layer are calculated from the theory of Danielli (Appendix A), and are rough values only. Nevertheless, the values closely parallel those found for the cells, both in magnitude and in variation from molecule to molecule. The permeability of the oil layer may be varied, for some molecules at least, by a factor of probably more than 100, if different oils are considered. It will be seen, however, that in certain cases, e.g. urea and glycerol, there is a variation

of at least 3000-fold in the permeability of different cells to the same molecule. Whether this large difference can be entirely accounted for by variation in the chemical character of the oil layer cannot as yet be stated.

TABLE XXII. PERMEABILITY OF DIFFERENT CELLS, ETC. IN MOLS PENETRATING PER SEC. PER μ^2 PER MOL PER LITRE CONCENTRATION DIFFERENCE.

All values have been multiplied by 10^8 , except the olive oil : water partition coefficients.

	<i>Ox crataegae</i>	<i>Achasia punctulata</i> unfertilised egg	<i>Chaetopterus</i> unfertilised egg	<i>Cumingia</i> unfertilised egg	<i>Chama cratophylla</i>	<i>Placochium denticulatum</i>
Trimethyl citrate	6.7	0.1
Anupurin	6.1	2.5
Propionamide	.	2.3	.	.	3.6	0.20
Acetamide	.	1.0	.	.	1.5	0.067
Urotropin	0.07	0.00025
Glycol	0.21	0.73	2.4	2.6	1.2	0.033
Methylurea	0.19	0.011
Urea	7.8	.	.	.	0.11	0.0036
2,3 Dioxyp propane	0.41	1.3	.	.	2.4	.
xy Dioxyp propane	0.11	0.67
Malonamide	0.0039	0.00083
Dioethylene glycol	0.075	0.43
Glycerol	0.0017	0.005	1.0	.	0.021	0.00033
Erythritol	0.0013	0.000069
Saccharose	0.0008	0.000008

	<i>Pyralis litoralis</i>	<i>Ceratomya rubradialis</i>	<i>Spirogyra</i>	<i>Rhoco discolor</i>	<i>Ceramium diaphanum</i>	<i>Melvinia</i>
Trimethyl citrate	.	0.24
Anupurin	.	0.61
Propionamide	1.4	0.22	0.45	0.16	2.8	3.0
Acetamide	0.28	0.08	.	.	.	0.47
Urotropin	0.031
Glycol	0.097	0.11	0.11	0.17	0.83	0.375
Methylurea	0.031	0.0089	0.028	0.0084	0.097	0.12
Urea	0.0083	0.0015	0.018	0.0028	0.083	0.042
2,3 Dioxyp propane
xy Dioxyp propane
Malonamide	0.0019	0.00036	0.0016	0.00022	0.025	0.018
Dioethylene glycol
Glycerol	0.0024	0.0018	0.0033	0.011	0.021	0.033
Erythritol	0.00012	0.00031	0.00042	0.0005	0.0021	0.013
Saccharose	0.000006	0.00008	.	.	.	0.0058

TABLE XXII (continued)

	<i>Bacterium paracoli</i>	<i>Reggiata mirabilis</i>	<i>Gregarina</i>	Olive oil- water partition coefficient	Permeability of 5 m μ of olive oil (calculated, Danielli)	Permeability of 5 m μ of water (calculated)
Trimethyl citrate	.	.	.	0.047	(170)	0.90×10^9
Antipyrin	.	.	.	0.032	(129)	1.05×10^9
Propionamide	.	.	.	0.0036	3.0	1.38×10^9
Acetamide	.	.	.	0.00083	0.83	1.79×10^9
Urotropin	.	.	.	0.00021	.	1.17×10^9
Glycol	.	1.39	0.67	0.00049	0.75	1.73×10^9
Methylurea	.	1.17	.	0.00044	0.058	1.61×10^9
Urea	0.083	1.58	0.25	0.00015	0.018	1.78×10^9
α Dioxyp propane	.	.	1.32	0.0057	2.6	1.60×10^9
γ Dioxyp propane	.	.	.	(0.001)	0.75	1.60×10^9
Malonamide	0.028	.	.	0.00008	0.0021	1.38×10^9
Diethylene glycol	.	.	.	0.005	0.485	1.45×10^9
Glycerol	0.055	1.06	0.018	0.00007	0.0051	1.44×10^9
Erythritol	0.005	0.84	.	0.00003	0.00007	1.24×10^9
saccharose	.	0.14	.	0.00003	.	0.75×10^9

When comparing different molecules diffusing through the same membrane it is preferable to compare values of $PM^{\frac{1}{2}}$, not of P , since in this way the purely mechanical factor of variation in molecular weight is eliminated. For example, values of P for ethylene glycol and $\alpha\gamma$ dioxyp propane usually differ significantly, but $PM^{\frac{1}{2}}$ values are often identical within experimental error.

Finally, one experimental defect in practically all of these studies must be pointed out. It is usually assumed that the structure of the membrane is not significantly affected by the concentration of non-electrolyte used in permeability studies, and that therefore the permeability constant obtained in all cases refers to the same independent membrane. This is an assumption which is probably true in most, but not in all, cases. Investigation is needed of the variation in P : (1) with different concentrations of the same non-electrolyte; (2) when one non-electrolyte penetrates in the presence of another.

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CHAPTER IX

PERMEABILITY TO GASES

By J. F. DANIELLI

THOUGH of obvious importance, the permeability of membranes to gases has been comparatively little investigated.

All tissues studied have a high permeability to gases, with the exception of the swim-bladder of fishes, which is practically impermeable to O_2 and N_2 (Bohr, 1893). Northrop has studied the permeability of dried collodion membranes to a large number of substances, including H_2 , N_2 , O_2 , CO_2 , HCl and NH_3 gases. Northrop believes that as the pore size in a collodion membrane is decreased, the importance of dissolution of the substances in the collodion becomes greater, and in the limiting case with membranes prepared with a minimal pore size this factor of membrane solubility is the dominant factor. The evidence he gives in support of this is conclusive; thus the permeability of a dried membrane is, for H_2 , 7.5; for N_2 , 0.45; for O_2 , 1.1; for CO_2 , 7.0 in $cm.^2 \times 10^4$ per day. If diffusion occurred through pores only, the rates should be as 7.5 : 1.7 : 1.6 : 1.4. Furthermore, even when the membranes are saturated with water, which should block the pores and reduce the permeability by some 10,000-fold, there is practically no change in the observed permeability. Clearly a partition coefficient is involved. When correction is made for this and the Fick diffusion constants are calculated, we find (Northrop) $D=75$ (H_2), 3.0 (N_2), 4.8 (O_2) and 1.5 (CO_2) in $cm. \times 10^4$ per day. If Q_{10} values were available, we should be able to calculate values of $PM^{\frac{1}{2}} Q_{10}^{(T+10)/10}$ and thereby see whether these molecules are diffusing through a homogeneous medium. If the medium proved to be homogeneous, the large variation of permeabilities would mean the viscous resistance encountered by these molecules varied considerably with different molecular species, although all molecular species can permeate all parts of the membrane to the extent indicated by the partition coefficients. If the membrane is not homogeneous, the variation in permeability may be due to some regions being permeable to some molecular species and not to others. In the case of silica

glass membranes, for example, Barrer (1934) has shown that H_2 is able to penetrate the whole crystal lattice, but that O_2 and N_2 can only penetrate through the spaces between the micro-crystals composing the glass.

An important study of the permeability of tissues was made by Krogh (1918). He gives values for the relative permeability of muscle tissue to oxygen, nitrogen, carbon dioxide and carbon monoxide which show that, per gram mol of dissolved gas, these various gases diffuse at roughly the same rate. Krogh also studied the relative permeability of a number of different tissues to oxygen. The permeabilities in c.c. of oxygen at N.T.P. passing through 1 cm^2 of material 1μ thick per atmosphere of oxygen at 20°C . are: water 0.34; gelatine 0.28; muscle 0.14; connective tissue 0.115; chitin 0.013; rubber 0.077. These values need to be multiplied by 7.2×10^3 if it is wished to obtain the permeabilities in terms of c.c. passing per gram mol per litre concentration difference.

Wright (1934) has measured the permeability of various tissues to CO_2 . In water, diffusing gases obey the relationship $DM^{\frac{1}{2}} = \text{constant}$, where D is the Fick diffusion constant. Wright states that O_2 and CO_2 conform to this equation in diffusing through connective tissue. He also shows that in certain cases the presence of HCO_3^- in physiologically important amounts does not contribute greatly to the permeability of CO_2 . Thus he finds that the permeability constant was independent of the pressure of CO_2 , which would not be the case if HCO_3^- contributed greatly to the transport of CO_2 . He also showed that the permeability to CO_2 of parchment soaked in Ringer is 1.35 (in Krogh's absolute units) and for parchment soaked in 0.15 M NaHCO_3 the permeability is 1.54, an increase of about 14%. Hill (1928) predicted that such an increase would be found. Wright argues that as frog tissues usually contain only about 0.015 M NaHCO_3 , the CO_2 carrying action of HCO_3^- will be correspondingly reduced to 1.4%, and is therefore of negligible influence in dealing with CO_2 permeability under physiological conditions. The permeabilities found for tissues were: frog muscle, 5.3; connective tissue (dog), 2.65; frog skin, 3.1, in the units of Krogh. There appears to be some possibility that the permeability of muscle to O_2 relative to that of CO_2 is less than would be predicted from the equation $DM^{\frac{1}{2}} = \text{constant}$ (Krogh, 1918; Hill, 1928; Wright,

1934. If this proves to be so, and Wright is correct in supposing that HCO_2 does not contribute substantially to CO_2 transport, then it must follow that the muscle membranes are more permeable to CO_2 than to O_2 . This is known to be true for red cells.

Hill (1928) has given a valuable theoretical treatment of the diffusion of oxygen in tissues such as muscle and nerve, which provides a basis for calculating approximately the degree of oxygen saturation, and the time taken to recover from an oxygen debt, etc. for tissues of various shapes and sizes. For a resting nerve to be fully supplied with oxygen, Hill obtained the formula $r_0 = \sqrt{4ky_0/a}$, where r_0 is the maximum radius for a nerve fully supplied with oxygen if the oxygen pressure outside the nerve is y_0 ; k is the diffusion constant of oxygen, and a is the rate of oxygen consumption in c.c. per gram per min. Using $a = 4.7 \times 10^{-4}$ c.c., $k = 1.4 \times 10^{-15}$, the values of the critical diameter for saturation given in Table XXIII were obtained. Thus, at 20°C . a nerve 3 mm. or less thick would obtain sufficient oxygen at rest in air. Ordinary cylinder nitrogen may contain 0.5% oxygen; the corresponding critical diameter is 0.49 mm. Obviously carefully purified nitrogen is needed to asphyxiate a small nerve. Hill gives a large number of other special cases which are of great value experimentally.

TABLE XXIII. CRITICAL DIAMETERS FOR COMPLETE SATURATION OF A NERVE WITH OXYGEN AT VARIOUS PARTIAL PRESSURES IN ATMOSPHERES

Pressure	1.00	0.21	0.10	0.010	0.001	0.0001
Diameter (cm.)	0.69	0.32	0.22	0.069	0.022	0.0069

Permeability of the Erythrocyte to Gases. The outstanding work on this aspect of erythrocyte permeability is that of Hartridge & Roughton (1927) by the method previously described (Chapter II). In Fig. 30 the combination of haemoglobin with O_2 is represented as a function of time for laked and unlaked cells, and the difference between the two curves is a measure of the rate of penetration of O_2 through the erythrocyte membrane. It is seen that the presence of the membrane retards the rate of combination of O_2 with Hb, so that the process is about ten times slower. In Fig. 31 the percentages of HbO_2 and COHb are shown as functions of time with the intact erythrocyte, and it is clear that O_2 penetrates the cell more rapidly than CO.

An interesting effect of calcium on the penetration of O_2 into the erythrocyte is claimed by Warburg (1911), who finds that the rate of O_2 consumption of young goose erythrocytes is decreased by addition of calcium to the suspension medium, whereas the rate of O_2 consumption of haemolysed red cells is unaffected by this metal.

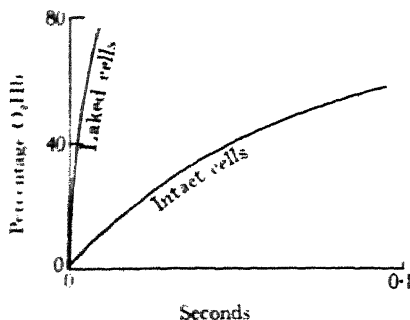


FIG. 30. Rate of combination of O_2 with *a* red cells, *b* laked red cells.

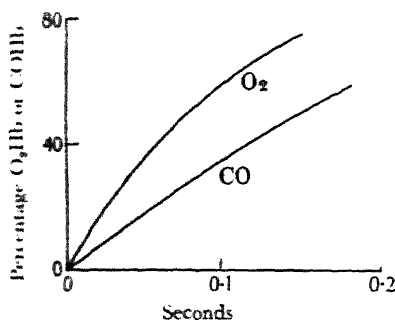


FIG. 31. Rate of combination of O_2 and CO with red cells.

Dirken & Mook (1931), using a technique which was essentially similar to that of Hartridge & Roughton, but differing in that the serum surrounding the cells was actually separated from them at definite times after mixing by a process of ultra-filtration, found that the decrease in the CO_2 content of the serum saturated with this gas, due to the penetration into the cells, occurs within 0.1 to 0.4 sec., but the rise in the HCO_3^- and the decrease in the Cl of the serum due to the "Hamburger Shift" is only within approximately 1.1 sec. Results similar to those

of Hartridge & Roughton on the penetration of O_2 into the cells were obtained. It would seem from the results of Dirken & Mook that the rate of penetration of CO_2 is more rapid than that of O_2 .

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CHAPTER X

PERMEABILITY TO WATER

By H. DAVSON

COMPARED with the amount of information regarding membrane structure which permeability studies of organic and electrolytes have yielded, that obtained from the study of the permeability of cells to water has not been very great; nevertheless, some very interesting facts have been brought to light regarding the effect of the ionic environment on the permeability of cell membranes by the study of the penetration of water. Apart from these facts, however, the accurate determination of the permeability constant of cell membranes to water has become of immediate importance with the development of quantitative methods of measuring the permeability of membranes to solutes which, as we have seen, involve a knowledge of the permeability constant to water (see Chapter IV).

The chief experimental methods, and the necessary equations for the determination of the permeability constant of a cell to water have already been given in some detail; the results of the measurements on Echinoderm egg cells, protozoa, plant cells, Annelid egg cells, Mollusc egg cells and of vertebrate fibroblasts, leucocytes and erythrocytes have been collated in a valuable paper by Lucké *et al.* (1939) and are presented in Table XXIV, modified from this paper. These authors point out that the relatively low permeability to water of Echinoderm eggs is not peculiar to marine eggs in general, but that a similar low order of permeability has been found for plant cells and for a protozoon. The great permeability of the erythrocyte to water (in the case of man thirty times greater than that of the *Arbacia* egg) is striking.

An interesting example of the faulty information which may be obtained from permeability studies when an inadequate equation is used for the analysis of the results is provided by three papers of McCutcheon & Lucké (1925-1927) in which experiments on the swelling and shrinking of *Arbacia* eggs are described and permeability constants are calculated on the basis of a simple "unimolecular" equation to which Lillie (1916) claimed that the

TABLE XXIV. COMPARATIVE PERMEABILITIES TO WATER
(TAKEN FROM LUCKÉ, HARTLINE & RICHES, 1936)

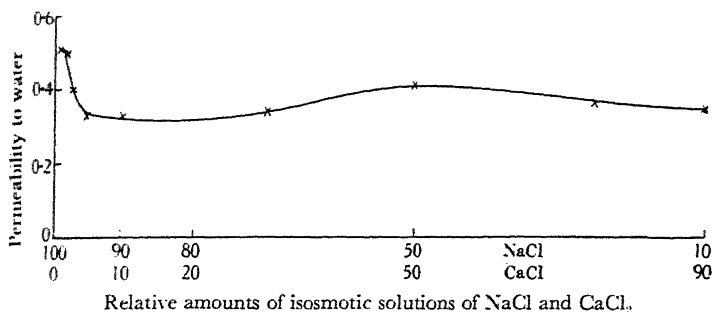
The constants used express the number of cubic micra of water which pass through 1 sq. micron of cell surface per min., per atmosphere difference in osmotic pressure, and (unless otherwise stated) at a temperature of 20° to 22° C. All values have been restricted to two significant figures			
	Kind of cell	Permeability	Measured by
Echinoderm (egg cells)	<i>Arbacia punctulata</i> (unfertilised)	0.1	Lillie (1916, 1917); Lucké <i>et al.</i> (1931)
	<i>Paracentrotus lividus</i> (unfertilised)	0.1	Maxia (1934)
	<i>Arbacia punctulata</i> (fertilised)	0.2-0.3	Lillie (1916, 1917); Mc- Cathcheon & Lucké (1932)
	<i>Paracentrotus lividus</i> (fertilised)	0.2	Maxia (1934)
Protozoa	<i>Strongylocentrotus</i> , <i>Dendroaster</i> , <i>Patiria</i> , <i>Pisaster</i> (unfertilised)	0.1-0.4	Leitch (1931)
	<i>Zoothamnium</i> sp. <i>Gregarina</i>	0.12-0.25 0.2	Kitching (1938) Adcock (1940)
Plant	<i>Fucus vesiculosus</i> <i>Allium</i> sp. <i>Salvinia auriculata</i>	0.16 0.3 0.55	Restühr (1935) Levitt, Scarth & Gibbs (1936) Huber & Hofer (1930)
Annelid (egg cells)	<i>Chaetopterus pergamentaceus</i>	0.4-0.5	Lucké <i>et al.</i> (1939)
Mollusc (egg cells)	<i>Cumingia tellonoides</i>	0.4-0.5	Lucké <i>et al.</i> (1939)
Vertebrate	Mouse, rat, chick	0.4-1.0	Brues & Masters (1936)
	Rabbit	0.3	Shapiro & Parpart (1937)
	Man	1.3	Shapiro & Parpart (1937)
	Ox	2.5	Jacobs (1932)
	Man	3.0	Jacobs (1932)

kinetics of swelling of these eggs conformed. McCutcheon & Lucké apparently demonstrated that the rate of penetration of water varied with the concentration of salts in the sea water; thus, if the swelling of eggs in 80‰ sea water was measured, a value for k of 0.07 was obtained, whilst with 20‰ sea water a value of 0.006 was calculated. Northrop (1927) derived a more suitable equation and showed that by using this to calculate permeability constants from McCutcheon & Lucké's results no dependence of the permeability constant on sea-water concentration, within wide limits, was present: this was later confirmed by Lucké *et al.* (1930). However, Leitch (1931), using a correct equation, has found a slight drift in the value of the permeability constant with varying salt concentration of the medium. The use of the more accurate equation enabled Lucké and McCutcheon to measure a difference in the permeability constant according as water entered endosmosis or left the cell (exosmosis), the latter being smaller than the former, giving a ratio of the constants in the two cases of 1.10. A larger difference was found by Adolph (1936) in the case of the worm *Phascolosoma*: the permeability constant for endosmosis was found to be 2.1 as against a value of 1.1 for exosmosis.

McCutcheon & Lucké (1928) found that the permeability constant of *Arbacia* eggs was considerably increased when the latter were allowed to swell in pure hypotonic dextrose solution, as opposed to hypotonic sea water; addition of calcium in a concentration of about 0.001 *M* brought the constant back to its value in sea water. Sodium and potassium when added to the non-electrolyte medium had no apparent effect, but when added in the presence of calcium they were found to antagonise the action of the divalent ion. Lucké & McCutcheon (1928), continuing this investigation, found that increasing the valence of the cation beyond 2 in the series of cobaltamine chlorides had no further effect in inhibiting the rate of penetration of water into the eggs when suspended in a non-electrolyte medium; if, however, sulphate which accelerates the penetration of water was added to the medium, it was found that the effect of the cobaltamine chlorides in decreasing the rate of penetration below that in non-electrolyte solution plus sulphate increased with the valency up to the 6 valent ion. Sulphate was not the only anion which caused acceleration, tartrate and citrate being also effective. In fact the valency rule was found to apply here, too; increasing

the valency of the anion produced a larger acceleration. The authors conclude that the antagonism observable between added salts consists in an antagonism between cation and anion, the former decreasing permeability and the latter increasing it. It should be remembered, however, that these authors were working in practically electrolyte-free solutions, a generally unsatisfactory medium for marine eggs.

Fukuda (1936) has studied ionic antagonism in regard to the permeability of marine eggs to water, and in this study the concentration of electrolytes has been maintained at a high level; the results on sodium-calcium antagonism are shown in Fig. 32;



16. 32. Effect of variation in the Na : Ca ratio on the permeability of eggs of *Anthocardis crassispina* to water.

these results were obtained with *Anthocardis crassispina*, and the eggs of *Pseudocentrotus depressus* were found to behave in the same way. It is seen that when the proportion of NaCl in the mixture is very high the permeability is also high; as the proportion of calcium is increased the permeability is depressed, but increasing the concentration of calcium beyond a certain point causes an increase in permeability again until the Na/Ca ratio is about unity; further increase in the proportion of calcium depresses permeability, so that a pure solution of calcium chloride gives a permeability to water not far removed from that in sea water. Rather similar effects were observed with sodium and magnesium.

The permeability of ox and human erythrocytes to water has been measured by Jacobs (1932) and the results are incorporated in Table XXIV. Jacobs & Parpart (1932) have studied the influence of salts on the rate of penetration of water into the ox

erythrocytes some of their results are presented in Fig. 33, where the time required to reach 75% haemolysis is plotted against the concentration of salts in the suspension medium; the amounts of salt present are small, so that their osmotic pressure is not an important factor; this is indicated by a curve which shows the influence of added sugar on the time of haemolysis. The curves

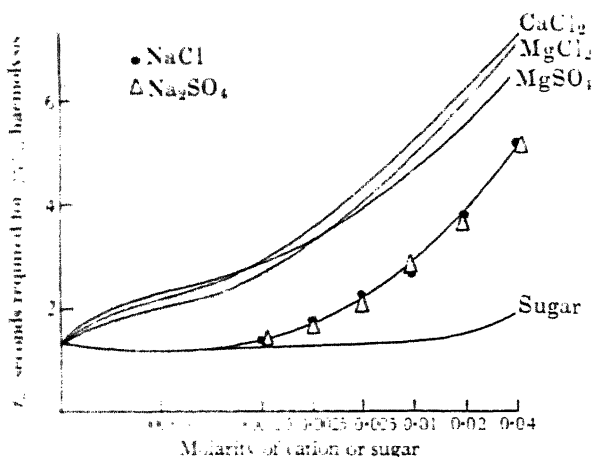


Fig. 33. Effect of salts on the time, $t_{0.75}$, taken by red cells to reach 75% haemolysis. The tonicity of the salt solutions is too low to affect the rate of penetration of water directly, as is shown by the curve with sugar, so that the variations in $t_{0.75}$ are due to effects on the cell membrane (modified from Jacobs & Parpart, 1932).

show that all of the salts studied decrease the permeability of the erythrocyte to water; calcium is, however, considerably more effective than sodium.

The leucocyte has been studied by Shapiro & Parpart (1937) and the permeability constant is found to lie between the high value of the erythrocyte and the low value of the *Arbacia* egg (Table XXIV). These authors observed that the rate of exosmosis of water from the cell is about four times greater than the rate of endosmosis. This result differs from that of Lucké *et al.* (1930) with *Arbacia* eggs, who found the rate of endosmosis to be slightly greater than the rate of exosmosis. Whether or not these differences in permeability constants, according as the water molecule is entering or leaving the cell, imply an asymmetry in the membrane

is difficult to say, since the cells are obviously under different experimental conditions in the two cases, even though these differences may not be very great.

Kekwick & Harvey (1934) claimed that the permeability of the *Arbacia* egg to water was reduced under anaerobic conditions; however, Hunter (1936) has shown that the differences found by Kekwick & Harvey were sufficiently small to lie within the normal range of variation of the permeability of different samples of eggs to water, and that the permeability was apparently uninfluenced by modifications of the oxygen tension of the medium.

The effect of fertilisation on *Arbacia* eggs was studied by Lillie (1916-1918), and it was shown that on fertilisation the permeability is increased about fourfold (see also McCutcheon & Lucké, 1932; Stewart & Jacobs, 1936); results with artificial fertilisation, e.g. with butyric acid, also indicated an increased permeability, but the results were irregular. A similar increase in permeability on fertilisation has been observed by Maxia (1934) with the egg of the sea-urchin *Paracentrotus lividus*.

Baptiste (1935) has studied the influence of ions on the permeability of potato and carrot disks to water. Soaking the disks overnight in hypotonic solution of the chlorides of potassium, ammonium and sodium produced an increase in permeability over the control, whilst the chlorides of magnesium and calcium decreased permeability. De Haan (1935) has shown that the permeability of the epidermis cells of onion scales to water may be affected differently by the same salt according as the concentration is varied. Thus calcium nitrate and $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ decrease permeability in low concentrations but increase it in high ones; sodium nitrate produced only acceleration. A possible increase in permeability to water due to mechanical stretching of the membrane is indicated by the exact determinations of Levitt *et al.* (1936) on onion-scale protoplasts.

Beck & Shapiro (1936) have made an interesting preliminary study of the nuclear membrane of the egg of *Asterias forbesii*, the results suggesting that the nuclear membrane has a permeability not less than that of the plasma membrane.

Gross (1940) has found that the protoplast of the plankton organism *Ditylum Brightwelli*, when transferred from sea water to isotonic NaCl, shrinks in a few seconds to a small fraction of its previous volume. This loss of water, and perhaps also loss of

salts, etc., occurs without any difference of osmotic pressure across the membrane. From unpublished results of Gross it has been calculated that the water loss may be as high as $2\mu^3$ per μ^2 per sec., a remarkably high rate and only compatible with a passage of water through a membrane of great porosity, or else with water excretion by a most efficient mechanism, perhaps of the contractile vacuole type. Further investigation of this phenomenon, particularly by impedance studies, should reveal valuable information on the modes of behaviour of the cell membrane.

Starling (1896) formulated the principles governing the passage of water through the walls of the blood capillaries. If the hydrostatic pressure at any given point in the capillary is greater than the difference in colloid osmotic pressure of the fluids on the two sides of the capillary wall, water is filtered out from the capillary into the tissue spaces. If the colloid osmotic pressure difference is higher than the hydrostatic pressure, fluid flows into the capillaries from the tissue spaces.

Landis (1927, 1934) has studied the permeability of the capillary wall to water, finding with the frog capillary a permeability of about $6\mu^3$ per μ^2 per sec. per atmosphere osmotic pressure difference. This is a value far greater than is found for any cell membrane, and the water flow in this case occurs mainly through pores in the capillary wall. The factors affecting permeability of the capillary wall have been reviewed by Landis (1934) and a quantitative analysis has recently been given by Danielli (1940).

Heavy Water. Since the discovery of the isotope of hydrogen, various studies on the biological influence of heavy water have been made. So far as permeability is concerned, Lucké & Harvey

(1935) have found no difference between the permeability constants of H_2O and D_2O penetrating into the *Arbacia* egg; these authors noted that D_2O is definitely injurious to the cell. Parpart

(1935) has shown that 99.5% pure D_2O penetrates the ox and rat erythrocytes at rates 44% less than the corresponding rates for H_2O ; this author failed to detect any injurious effect of D_2O on the red cell. Brooks (1935) has found similar results using sheep erythrocytes, but argues that the difference in apparent permeability may really be ascribed to an initial tendency for H_2O molecules to pass out of the erythrocyte into the surrounding hypotonic D_2O owing to the difference in fugacity. In the case of the different experimental conditions under which the *Arbacia* egg's permeability was measured, Brooks argues that it is quite

possible that no such difference of fugacity would have been present and consequently no initial movement of D_2O out of the cell; thus the failure of Lucké & Harvey to detect a difference in penetration rate would be accounted for.

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CHAPTER XI

PERMEABILITY TO PROTEINS AND TO LARGE LIPOID MOLECULES

By J. F. DANIELLI

1 *Permeability to Proteins*

GENERALLY speaking, the plasma membrane is supposed to be impermeable to proteins, though in certain cases, as in the case of enzymes and the penetration of viruses, proteins must pass through the membrane in some manner. Penetration of the plasma membrane by a protein molecule containing many polar groups seems unlikely from a qualitative point of view. It is of interest to calculate the maximum possible rate of penetration.

This has been done in two ways, assuming that the plasma membrane is a thin lipid layer (Danielli, 1940*b*). In the first case we can assume that the protein molecule diffuses into the plasma membrane in just the same way as small molecules do. This gives us the maximum rate of penetration of a small protein such as ovalbumin, of a single molecule penetrating 1 cm.² of membrane in 10^{450} years, per gram mol of protein per litre concentration difference. This rate is far too low to be of practical importance even for the infection of cells by viruses. In the second case we can assume that the protein molecule penetrates only when a molecule has sufficient kinetic energy to knock a hole in the plasma membrane large enough to allow penetration of a protein molecule. This gives a maximum rate of one molecule per cm.² in 10^{32} years—again a negligible rate, even for infection by viruses.

These are the only two ways in which a protein molecule may pass through a thin lipid layer by simple diffusion. Consequently, we can assert with some confidence that when proteins penetrate into the interior of the cell, they must do so through some specially differentiated part of the cell membrane. In view of this, it is interesting that viruses are supposed to infect a plant only when they can reach the protoplasm through the surface of a damaged cell, and that infection of healthy cells is supposed to occur via the protoplasmic connections between adjacent cells.

In the case of porous membranes, the passage of proteins is

determined mainly by pore size. The glomerular membrane of the kidney appears to be permeable to all proteins of the same or less diameter than red cell haemoglobin Bayliss *et al.* 1933. The average diameter of the protein-permeable pores of this membrane thus lies between 4 and 5 $m\mu$, and since serum albumin never appears in normal urine there are normally no pores as large as 6 $m\mu$. The frog's capillary membrane has a rather greater pore size, allowing slow passage of serum albumin and possibly, to a lesser extent, serum globulin. In the absence of platelets, ovalbumin, serum albumin and haemoglobin penetrate freely, and so do the smaller of the particles of gum acacia (Drinker, 1927; Saslow, 1938; Danielli, 1940*a*). In the presence of platelets, penetration of these substances is greatly restricted. The average diameter of the protein-permeable pores in the perfused frog is about 6 $m\mu$ or less in the absence of platelets, and in their presence a few pores of this diameter persist.

The permeability of both the glomerular and the capillary membranes to proteins is greatly increased by oxygen lack and by respiratory poisons. The action, however, is not on an active transport system; the increased permeability arises from the response of the cells forming the membranes, which behave in such a way as to allow the pore size to increase. Thus Krogh (1929) and Landis (1927, 1928) have shown that oxygen lack and respiratory poisons cause capillary dilatation accompanied by greatly increased permeability to proteins. Starling & Verney (1925) have found similar behaviour with the glomerulus. Krogh has advanced the view that dilatation and increase in permeability are inseparably linked. This may be so, but on the other hand increase in permeability may occur without dilatation if the blood platelets are removed (see Danielli, 1940*a*, re Saslow, 1938). The factors affecting permeability of the capillary membrane to proteins are therefore (1) pore size, (2) particle size. The first of these factors may be varied by dilatation and contraction, by the presence or absence of platelets, and by foreign proteins or polypeptides. The degree of dilatation is partly controlled by innervation, partly by hormones in the blood. Foreign proteins and polypeptides may displace other adsorbed proteins from the walls of a pore, becoming adsorbed themselves instead. Then, if the diameter of the foreign protein body is less than that of the original adsorbed protein, the capillary pore size will be increased,

and consequently so will the permeability to proteins. This phenomenon is used to increase permeability of collodion membranes to proteins. A collodion membrane with a pore size slightly greater than that of the protein which it is desired to filter may be quite impermeable to the protein in dilute salt solution, due to the blocking of the pores of the membrane by adsorption of protein. But, if higher polypeptides of small diameter, such as are found in Hartley's broth, are added, adsorption of protein is restricted and penetration will occur. This phenomenon may be responsible for the increase in capillary permeability to proteins found when higher polypeptides are present, and for the appearance of serum albumin in the urine when ovalbumin is present in the blood stream. It may also be responsible for many of the toxic albuminurias occurring in "kidney disease". On the other hand, it is possible that the action of the polypeptides is chemical. The relative importance of the physical and chemical actions has yet to be elucidated.

The permeability of capillaries to colloids is affected by many drugs. Very important papers dealing with the relationship between the action of certain drugs and the occurrence of "shock" are those of Dale & Richards (1918) and Dale & Laidlaw (1919). Histamine causes dilatation and increased permeability to colloids. Adrenaline has the reverse action. See also Feldberg & Schilf (1930). The action of drugs, however, varies markedly from species to species.

The walls of the intestine also appear to be slightly permeable to proteins. This is shown by the albuminurea which may follow feeding of ovalbumin, and by the reaction of allergic patients to feeding with proteins to which they are sensitive. The route of absorption of these proteins is unknown; it may occur via pores into the capillaries and particularly into the lymphatics; it may also occur by ingestion by the leucocytes which wander between the walls of the intestine and the intestinal contents, or even follow mechanically in the wake of these leucocytes in their wanderings through the walls of the capillaries and lymphatics. Verzar (1936) gives a more detailed discussion of the experimental observations.

{ A fascinating problem, at present unsolved, is propounded by the action of protein hormones such as insulin and secretin. Are they able to penetrate cell membranes, or do they act upon the surface of cells?

2. *Permeability to Large Fatty Molecules*

As typical molecules we may take stearic acid, tristearin, cholesterol, lecithin and carotene. And for typical membranes the intestine, the capillary, and cell plasma membranes.

In diffusing across cell membranes these types of molecules can readily penetrate into the lipid layer of the membrane, and in most cases diffusion across it is probably relatively rapid. But to diffuse out of the lipid layer into an aqueous phase is more difficult, and in many cases this probably constitutes the dominant barrier to free diffusion. In order to get out of the lipid layer kinetic energy of at least 500 to 1000 calories per CH_2 group is needed;* this amounts to perhaps as little as 8000 calories for stearic acid, but for a molecule such as tristearin may be as high as 60,000 calories or more. Stearic acid may therefore be able to diffuse relatively rapidly into, across and out of the cell membrane. But for a molecule such as tristearin diffusion out of the membrane is probably practically impossible. In addition some fatty molecules may not be able to move out of the membrane if in the plasma membrane there are molecules forming specific complexes, such as the complex between cholesterol and digitonin, or between fatty acids and bile salts, since such complex formation will raise the minimum energy necessary before a molecule can diffuse out of the membrane.††

It must therefore be considered probable that molecules such as tristearin, and perhaps the sterols, if they penetrate the plasma membrane, do so by some special process. For example, it is well known that if a monolayer of fatty molecules consisting of two different molecular species is compressed, in some cases one molecular species will be squeezed out of the monolayer into the underlying aqueous phase. A film which would otherwise be completely stable may thus be separated into its two components by a comparatively small pressure. The component which is squeezed out may then show spontaneous formation of micelles, which can diffuse elsewhere, or may enter into combination with proteins, or other cytoplasmic colloids. Such "squeezing out" forces may readily be exerted on the plasma membrane by general movements of the cytoplasm.††

* The correct figure is probably of the order of 2500 calories per CH_2 group, but a lower figure has been used so as to obtain the maximum possible rates of penetration.

In the intestine tristearin penetrates partly as neutral fat, passing by the lacteal-lymphatic route to the fat depots, and partly, after hydrolysis to stearic acid and glycerol, passing to the liver via the portal vein. Stearic acid exists in the intestine as fatty droplets, in micelles largely in combination with bile acids, and to a very minor extent as individual molecules. The concentration of individual molecules must be very low, for the maximum solubility of stearic acid as individual molecules is about one part or less in 10^8 of water, i.e. roughly $10^{-7} M$ or less. These individual molecules, contrary to the remarks of Verzar, will be almost completely ionised at pH 6.7, the pH of the intestine. At the surface of the micelles and droplets, the pK of fatty acids is displaced (Hartridge & Peters, 1922; Peters, 1931; Danielli, 1937), and at pH 6.7 the degree of ionisation will lie between 5 and 50%. This, however, may be diminished by complex formation between fatty acid and bile salt. Thus a fatty acid molecule may present itself to the plasma membrane of the mucosal cells as either an ionised molecule (RCO_2^-) or as a neutral molecule RCO_2H . In practice it is found that the rate of penetration is negligible unless the fatty acid is emulsified by bile salts. This is probably because the solubility of the fatty acid is so low that the number of molecules diffusing into the intestinal mucosa cell membranes is negligible unless the fatty acid is emulsified. Both the ionised and neutral molecule will readily be able to penetrate into the plasma membrane of the cells of the villi, and the molecule will become oriented at the external oil-water interface with its polar group (CO_2^- or CO_2H) in the aqueous phase, and its hydrocarbon chain ($R = C_nH_{2n-1}$) dissolved in the lipid layer of the membrane. If the fatty-acid molecule has reached the plasma membrane as a molecular complex with a bile salt, the complex may be broken down, so that the bile salt is free to act as a carrier for further fatty-acid molecules. In addition other unknown mechanisms may convey fatty acids into the mucosa cells. Beyond this point there is no evidence; the process by which fatty acid moves from the external surface of the plasma membrane to the interior of the cells is quite unknown. It may, however, be said that diffusion across the interior of the membrane is probably also quite rapid, but that diffusion out of the membrane into the aqueous phase of the mucosa cell is a more difficult process, due to the difficulty of

removing the hydrocarbon portion of the molecule from the fatty layer. Similar remarks apply to the other fatty molecules. There is some evidence (Verzar, 1936, discusses this) that a phosphorylation mechanism comes into action at some point in the process of transfer of fatty acid from the gut to the lymph, but at what stage is by no means clear. It is possible that the fatty acids, sterols and carotinoids move out of the membrane of the intestinal cells into the interior of the cells by simple diffusion. For example, a rat with an intestinal villi area of 450 cm.^2 can absorb about 3.5 gm. of triolein daily. This is equivalent to 10^{-4} gm. mols of fatty acid per cm.^2 per day. From the equations given by Danielli Appendix A), one can calculate that the maximum possible permeability of a cell membrane to oleic acid will probably lie between 10^{-2} and 10^{-5} gm. mols per cm.^2 per day, if the concentration of fatty acid in the fatty layer of the membrane is kept at 1 gm. mol per litre. This concentration is a perfectly possible value, so that the observed rate of absorption may possibly be due to simple diffusion across the cell membrane. This appears the more probable in that inhibition of phosphorylation does not prevent penetration of fatty acid into the cells—it merely stops synthesis of this fatty acid into neutral fat Jeker, 1936; Verzar & Jeker, 1936).

The mechanism whereby neutral fat passes into the capillaries Frazer, 1938) is quite unknown. It is quite certain that it does not do so by simple diffusion of single molecules.

In the absorption of sterols from the intestine a much more specific permeability is exhibited. Cholesterol is absorbed fairly rapidly, but allocholesterol, the four isomeric dihydrocholesterols and the plant phytosterols are not absorbed at all (Schönheimer, 1929, 1932). All these substances are emulsified by bile salts and should readily penetrate into the plasma membranes of the absorbing cells. Beyond this point we are again ignorant. Verzar (1936) is of the opinion that esterification of cholesterol may be an essential step in the movement of cholesterol, and that other sterols may not be absorbed because they cannot be esterified by the cell enzyme systems. In addition there are fairly marked differences in the behaviour of sterols in monolayers (see particularly Adam *et al.* 1935) which may assist in the process of differentiation.

Lecithin appears to be broken down into its components in the intestine and absorbed as such. Carotene possibly penetrates

by simple diffusion, since it has been found that, for example, 50-100 mgm. of *n*-hexadecane may be absorbed daily compared with several grams of neutral fat, and hexadecane cannot enter into chemical reactions and consequently must be absorbed by a physical process. So far as is known such substances as phytol and oleyl alcohol also penetrate by simple diffusion (Channon *et al.* 1926, etc.).

On the permeability of the blood capillary membrane to fats, comparatively little work has been done. A most interesting indirect study is that of Süllman & Verzar (1934) on the apparent particle size of the fat in serum. Serum is known to contain part of its fat as droplets, but part of it exists in a colloidal form not visible under the microscope. This was studied by observing the relative rates at which water blue (diameter $1.2\text{ }\mu$), congo red (diameter $2\text{ }\mu$), serum proteins (diameter $6\text{ }\mu$ or more, and blood fat pass through collodion membranes of varying permeability. The results are shown in Table XXV. Membranes which are permeable to protein appear also to be permeable to part of the serum fat. As the capillary membrane is permeable to protein, it is presumably also permeable to part of the serum fat. Whether this fat, which is free to diffuse through the pores of a porous membrane, is in the form of fat micelles or of fat-protein complexes is not known.

TABLE XXV. PERMEABILITY OF GRADED COLLODION MEMBRANE TO DYES, SERUM PROTEIN AND SERUM FAT

Pore size increases from A to F. - denotes membrane permeable

Membrane	Water blue	Nile blue	Protein	Diffusible fat as % of fat in serum
A				
B				
C				
D				2-8
E			+	10-26
F				24-27

It is clear from what has been said that of the permeability of natural membranes to fats and proteins very little is known, despite the physiological importance of these compounds.

It is quite possible that in the past far too little attention has been given to the possibilities of phagocytic or amoeboid activity. It is well known that in the lower animals, e.g. sea anemones, digestion is very largely carried out by phagocytic action. The cells of the higher animals are usually regarded as lacking in such

activity, but it must be remembered that, in tissue culture, the majority of cell types show that they retain the capacity for motion. It is far from improbable that most cells also retain the capacity for phagocytic action, if only in a modified, specialised or restricted form. Such a capacity would be most important in the activity of the intestinal mucosa, in the passage of fat from and into the fat depots, and in the movements of protein molecules, etc. Many workers incline to the view that the restriction of absorptive activity in e.g. the intestinal mucosa, and in kidney tubules by poisons which act upon phosphorylating systems, is due to a general poisoning of cellular activity, and is not due to the absorption mechanism involving phosphorylation of the absorbed molecules. Such contentions would gain force were it demonstrated that phagocytic mechanisms are of importance in absorption. It is fairly certain that such phagocytic activity would depend upon the metabolism of phosphorylated sugars for its ultimate source of energy, and hence would be paralysed by phosphorylase poisons.

Recently Chambers & Kopac (1937) have shown that fat droplets may pass spontaneously from the exterior of a cell to the cell interior. This passage appears to be dependent upon the fat droplet having a high oil-water surface tension, of the order of 9 dynes/cm. in the case of the cells studied by Chambers & Kopac. It therefore seems improbable that this mechanism can be important for the movements of fat particles which have passed into the blood stream or into cells, for all such fat particles are in contact with protein and will have a surface tension probably less than 1 dyne/cm. Nevertheless this mechanism should not wholly escape our notice, and in the case of absorption from the intestine its investigation may prove very profitable.

To conclude, it may be said that students of absorption from the intestine and of intermediary metabolism have in the past relied too much on the classical methods of biochemistry, and have failed to analyse the physico-chemical processes which are an integral part of absorption processes. This failure in turn may have led to too much responsibility being implicitly delegated to these physico-chemical processes, and insufficient attention being paid to the cell as an active organ. The investigation of absorption problems, with due attention to the full biological activity of the cell, may well prove most advantageous.

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CHAPTER XII

PERMEABILITY OF ERYTHROCYTES TO IONS

By H. DAVSON

PERMEABILITY OF RED CELLS TO ANIONS

THE history of the emergence of the definite view that the erythrocyte is specifically permeable to anions, e.g. Cl^- and HCO_3^- , is interesting in that it demonstrates how complicated the equilibria of the erythrocyte appeared at a time when the Arrhenius theory of electrolytic dissociation (1887) had not been put forward. Zuntz (1868) and Schmidt (1867), working independently, found that on passing CO_2 into blood the titratable alkalinity of the serum increased far more than when serum is treated alone. The phenomenon of the "Chloride Shift" usually associated with the name of Hamburger was discovered by Nasse (1878), who found that chloride passed from serum to cells on bubbling CO_2 into blood. Hamburger (1891) found that NO_3^- exchanges for Cl^- between cells and serum and also rediscovered the observation of Nasse on the chloride shift with CO_2 , and noticed an increase in the percentage of solids in the serum after treatment of the whole blood with CO_2 . The general theory at this time as to the cause of the increase in the titratable alkalinity was to the effect that CO_2 combined with part of the base initially combined with protein in the cells, and the product, K_2CO_3 , passed out into the cells in exchange for KCl .

However, Gürber (1895) found no significant change in the Na and K contents of cells and serum after treatment of the blood with CO_2 , and in consequence of this discovery Koeppe (1897) postulated that only the HCO_3^- ions, liberated by combination of CO_2 with the potassium haemoglobin in the cells, diffused out of the cell in exchange for Cl^- from the serum, and specifically made the assumption that the erythrocyte membrane was permeable to anions.

Hamburger (1893) was able to demonstrate a difference in the Cl^- content of arterial and venous plasma due to the difference in tensions of CO_2 in the two fluids.

The view of Koeppe that the erythrocytes are permeable to Cl^- has been amply confirmed since; thus as early as 1902 Hamburger & Von Lier demonstrated that SO_4^{2-} and NO_3^- may be exchanged for Cl^- in the cells; in an indirect way they showed that L. Br. lactate, salicylate, oxalate, phosphate, arsenate and borate also penetrate the membrane. Falta & Richter-Quittner (1919), however, claimed that *undamaged* erythrocytes were impermeable to Cl^- and also Straub & Meier (1919), claimed that the cell was permeable to anions only on the acid side of pH 6.7; again, Hober (1922) quotes some results of Petow, from his laboratory, showing that blood is sometimes obtained which is completely impermeable to anions. In view, however, of the more recent results of Doisy & Beckman (1922), Henderson (1928) and Dirken & Mook (1931), to choose just a few, on the distribution of Cl^- and HCO_3^- in the blood *in vivo* and its variation with CO_2 tension, the claims of Falta & Richter-Quittner and of Straub & Meier must be put aside.

The literature contains many studies of anion permeability in respect to the erythrocyte, but it is unfortunate that almost without exception they are only of a semi-quantitative nature, and in many cases the workers have not been careful to distinguish a permeability process from an equilibrium condition.

Wiechmann (1921) has made the claim that the rate of exchange of Cl^- with Br^- is influenced by Ca. Thus, whilst without Ca in the suspension medium, he found a final ratio of bromide in cells : bromide in suspension medium of 1 : 2.6; with Ca present the ratio was 1 : 6.2. These results are difficult to interpret, as it would seem that Wiechmann was not, as he thought, measuring the rate of penetration of Br into the cells; he allowed 2 hours of suspension in the bromide solution before separating the cells, and it may definitely be stated that exchange of Cl^- for Br^- is over within perhaps as short a time as 1 min. During the course of the experiment he bubbled O_2 through the cell suspension in order to remove CO_2 ; this treatment of course causes the cells to become alkaline and, by increasing the base bound by the haemoglobin, causes the ratio of anion in cells : anions in serum to become smaller. This explains his normal ratio of 1 : 2.6. A close inspection of his results for Ca shows that he most probably used his earlier experiments on a different batch of cells as controls, so that in view of the unsatisfactory nature of the

procedure it may be accidental that he observed the low ratio of 1 : 6 in the presence of Ca; at any rate the phenomenon seems definitely not to be an influence of Ca on permeability and would bear reinvestigation.

Ege (1922), using the change in volume of cells in isotonic solutions of ammonium salts as an index to the rate of penetration of the anions, on the assumption that ammonium salts penetrate as NH_4^+ and A^- , gives the relative values for the rates of penetration of a number of anions.

He states that tartrate and citrate do not penetrate. However, Ege ignores the possibility that polyvalent anions will produce a smaller increase in volume of the erythrocyte than monovalent ones, so that his figures are only of use in comparing anions of the same valence; in the case of the ammonium salts of weak acids, it seems highly probable that the mechanism of penetration involves the penetration of NH_3 and the undissociated acid, so that the method is quite inapplicable to these substances. The penetration of an ammonium salt will also produce pH changes in the cell which will modify its volume, so that in the light of all these considerations it seems very doubtful whether Ege's figures mean a great deal.

Mond & Gertz (1929) have measured the rate of exchange of Cl^- with SO_4^{--} across the erythrocyte membrane for a number of different species; these authors used a direct chemical technique and plotted curves showing the rate of exchange so that, as far as they go, their results are of a reliable nature. The rates of exchange were found to vary with the species of erythrocyte concerned and were in the order:

man > pig > horse > goat > sheep > ox.

They compared these rates with the rates of penetration of erythritol into the same cells and obtained the same series:

man > pig > horse > goat > sheep > ox,

and concluded that both anionic exchanges and the penetration of lipid insoluble substances such as erythritol occur through pores.

The experimental material presented, however, is, in the present writers' view, entirely inadequate to maintain such a position. Thus only SO_4^{--} of all the possible anions has been studied; also

only erythritol of the lipoid insoluble non-electrolytes, yet it has been seen that certain species show quite irregular permeabilities to given substances, e.g. the mouse in the case of erythritol and mannitol, and it seems very likely that if Mond & Gertz had chosen another substance than erythritol an entirely different series of rates of penetration would have been obtained. Incidentally, figures on the permeability of a slowly penetrating substance like erythritol based on the haemolysis technique, as those quoted by Mond & Gertz were, may be unreliable in view of the recent work of Davson (1939) on the escape of electrolytes from the erythrocyte in non-electrolyte solutions.

The questionable logic of Mond in respect to his pore theory is demonstrated by his interpretation of his results (1930) on the $\text{Cl}^- - \text{SO}_4^{2-}$ exchange in the goose erythrocyte. He found this exchange to occur more rapidly than with human cells, yet goose cells are not permeable to glucose, whereas human cells are. Therefore, says Mond, glucose does not penetrate through a pore but by some other mechanism.

Ørskov (1933), using chlorine and iodine electrodes, claimed that the $\text{Cl}^- - \text{HCO}_3^-$ exchange occurred within 15 sec. and that the $\text{I}^- - \text{HCO}_3^-$ exchange occurred within 7 min.; the figure of 15 sec. is large compared with the value of less than 1 sec. obtained with another method by Dirken & Mook (1931). At any rate, results obtained with these electrodes must be interpreted with caution, as it seems to the writers very doubtful whether they are suitable for biological conditions, especially as Ørskov (1934) has changed his mind in respect to some of the observations described in the earlier paper.

Maizels (1934) has studied the permeability of the human erythrocyte to some forty different anions. Maizels placed cells with their normal HCO_3^- and Cl^- contents into solutions containing equivalent quantities of KCl and the K salt of the second anion to be investigated. After an interval of 5 min., the suspension was centrifuged and the cells analysed for Cl^- and sometimes for the anions studied; usually, however, the quantity of anion penetrating was obtained by difference. The ratio B/Cl , where B is the amount of second anion in the cells and Cl^- the amount of chloride, was used as a measure of the difference in the rate of penetration. As Maizels (1937) pointed out, this ratio B/Cl is to the relative rates of penetration of B^- and Cl^- , and

is of value only in comparing different second ions B^- with one another. The results of Maizels' experiments appear eminently reasonable and consistent with other data on molecular permeability. He found that the inorganic anions penetrate in the following order: $\text{CNS}^- > \text{I}^- > \text{NO}_3^- > \text{Cl}^- > \text{SO}_4^{2-} > \text{H}_2\text{PO}_4^-$. With aliphatic anions such as acetate, propionate, etc. he found that the rate of penetration increased with the size of the non-polar part of the molecule. With hydroxy- and keto-acids he found that the OH or CO group slows the rate, and the more effectively if in the α position than in the β position. He claimed that oxalate penetrates more rapidly than Cl^- ; but malonate and succinate more slowly. Substitution of $-\text{OH}$ or $-\text{NO}_2$ groups in the nucleus of benzoic acid increased the rate of penetration.

Timm (1937) has measured the escape of chloride from pig erythrocytes suspended in isotonic solutions of sulphate, phosphate, oxalate, lactate, aceto-acetate and hydroxy-butyrate by means of the chlorine electrode; this author has failed to distinguish between differences in rate and differences in equilibrium position, and from the curves presented it is not possible to distinguish between the relative rates at which the exchanges occur.

Höber (1936), using the ammonium salt technique of Ege, has studied the penetration of organic anions; it must be remembered that this technique is probably rather inadequate for the study of the anions of weak acids and it is likely that the results are due to penetration both as anion A^- and as free acid HA . Höber observed striking species differences; thus the ammonium sulphates of benzene, toluene, naphthalene and naphthylamine would not penetrate the erythrocytes of the ox and sheep, whilst in the case of human and mouse erythrocytes their penetration was more rapid than that of sulphate. With aliphatic fatty acids the rates of penetration of the ammonium salts were more rapid than that of sulphate and increased with the length of the hydrocarbon chain. With aromatic acids salicylate penetrated more rapidly than benzoate and phenylacetate. The ammonium salts of succinic, tartaric, fumaric and citric acids did not penetrate the erythrocytes of all the species examined, glycolate penetrated at about the same rate as acetate; lactate and pyruvate more slowly.

The criterion of penetration was the observation of haemolysis and the method used was not a sensitive one for measuring small

initial amounts of haemolysis, so that it is not impossible that, where impermeability is claimed, in fact some penetration did occur.

In opposition to Hober's results, Dziemian (1939) claims that, with all the species examined by him (cat, sheep, dog, rat, ox, rabbit, monkey) ammonium benzoate penetrated more rapidly than ammonium salicylate; further, the species relationships claimed by Hober in regard to the penetration of the ammonium salts of lipid soluble acids were also not found to be constant but varied with the salt studied. No correlation between permeability and lipid content of the erythrocyte was found by Dziemian.

According to Keilin and Mann (1941), when red cells are washed with acid phosphate buffer, their permeability to anions is lost.

This review of the published work on anion permeability in the erythrocyte reveals the unsatisfactory nature of our knowledge with respect to this branch of ionic permeability; thus, quantitative measurements are almost completely lacking, so that it is im-

possible to state whether the interesting ion thiocyanate penetrates, for example, ten or a hundred times more rapidly than the chloride ion, and similar remarks apply to practically all the other anions mentioned here.

PERMEABILITY OF RED CELLS TO CATIONS

In the earlier chapters of this book the erythrocyte has been treated as a salt-impermeable cell, and this impermeability has been specifically ascribed to an impermeability to cations, whilst anions have been assumed to penetrate freely. The work described on the behaviour of the erythrocyte to anions leaves no doubt that the latter is permeable to this species of ion; it follows then, as we have seen in Chapter III, that, unless secretory activity is involved, the erythrocyte must necessarily be impermeable to at least one of the cations Na and K if it is to maintain its integrity as a cell for any length of time.

Recently Davson (1940*a*) has shown that the cat erythrocyte is permeable to both potassium and sodium, and this, therefore, would seem to suggest that a cell may be stable under these conditions in spite of the considerations mentioned earlier. This would be possible if the plasma surrounding the cell contained substances which could not diffuse into the erythrocyte in a total

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concentration equal to the calculated difference in osmotic pressure due to the unequal distribution of salts and protein (about 0.01 *M*). It is doubtful, however, whether the blood plasma does actually contain this quantity of indiffusible substances.* The work of Davson consists of *in vitro* studies, so that there may be some specific factor in the blood of the intact animal which inhibits the penetration of one or both of these ions (see, however, Robinson & Hegnauer, 1936).

Apart from the above-mentioned difficulty in postulating an erythrocyte permeable to cations, there is a further one in that the erythrocytes of practically all species contain a much higher concentration of potassium than that in their surrounding medium (Table XXVI); consequently a metabolic activity must be further postulated to account for the maintenance of this concentration gradient.

TABLE XXVI. THE SODIUM AND POTASSIUM OF THE ERYTHROCYTES OF DIFFERENT SPECIES EXPRESSED AS MILLIMOLS PER 1000 GM. KERR, 1937)

	Potassium	Sodium
Rabbit	99	16
Rat	100	12
Man	110	.
Monkey	111	.
Pig	100	11
Horse	88	.
Goose	90	7
Guinea-pig	105	15
Sheep	18	84
	64	16
	58	46
Ox	22	79
Dog	9	107
Cat	6	104

Investigations on cation permeability in the erythrocyte may be divided into two classes, that in which the work has been designed to show whether the erythrocyte is under normal conditions permeable to cations, and a second class in which the more general problem of specific ionic permeability is envisaged; in this latter class of work the investigator is not greatly concerned with the behaviour of the erythrocyte in respect to its specific physiological functions but rather an apparent impermeability to

* Glucose penetrates very slowly, so that if glucose on penetration into the red cell is immediately metabolised, at least half of the osmotic pressure necessary may be made up by the blood sugar.

cations under normal physiological conditions is tacitly assumed and attention is focused on the mechanism whereby this impermeability is maintained, i.e. whether a metabolic process is concerned or, if not, with what special characteristic of the membrane can the impermeability be associated. The work belonging to the former class consists chiefly of *in vivo* studies, and in view of the limited view-point of this sort of investigation and the inability adequately to control the environment of the cells conditioned by the *in vivo* nature of the work, this class need not be described in detail.

Doisy & Eaton (1921) and Wakeman *et al.* (1927) concluded from their studies that the erythrocytes of man are impermeable to cations. They varied the cation concentrations in the plasma. Eisenmann *et al.* (1937) and McCance (1937) show results indicating the opposite view-point. Kerr (1926) has shown that injection of large quantities of saline or glucose solution into the dog causes cation exchanges between the erythrocytes and the plasma, and Yannet *et al.* (1935) find no shift of cations across the erythrocyte membrane in the case of the monkey and rabbit, whilst with the dog they find definite evidence of a movement of sodium. These authors point out that in general species with a high Na content in their cells, e.g. dog, ox, sheep, permeability to cations seems to occur. In conformity with this view, we have the findings of Robinson & Hegnauer and Hegnauer & Robinson (1936), who provide unmistakable evidence of the shift of cations across the membrane of the cat erythrocyte, which contains chiefly sodium; the more recent work of Cohn & Cohn (1939), where the radio-active isotope technique was used, which demonstrates a ready permeability of the dog erythrocyte to sodium; and that of Hahn *et al.* (1939) with the same technique, showing a very small permeability of the rabbit erythrocyte to potassium.

The work belonging to the first class, then, indicates that in certain species, notably the cat, sheep and dog, a change of the sodium and potassium content of the serum is followed by adjustments in the cells indicating a permeability to one or both of these ions; the evidence in respect to other species is conflicting. However, many *in vivo* studies cannot be considered to be very reliable in this instance owing to the possibility of the liberation of toxic factors into the blood, as a result of the sometimes rather drastic methods of inducing changes in the plasma content of

sodium and potassium. Further, there is no control of the cells experimented upon; thus the sudden liberation of new cells from the spleen into the blood stream during the course of the experiment would produce changes in the observed cation contents of the erythrocyte if these new cells had not the same average composition as those already in circulation. Again, a change in the volume of the cells by the migration of water will produce a change in the apparent cation content, since a given volume of cells will now contain a greater or smaller number of cells than before the water exchange, and unless this change in the water content of the cells is accurately allowed for (and this is a matter of some difficulty) illusory results may be obtained. Thus McCance's alleged changes in the potassium content of human erythrocytes, when the subject is suffering from pronounced salt lack, actually rely on determinations of the haemoglobin content of the blood and only secondarily on the chemical analysis of potassium, so that the 10% change he obtains may partly be covered by errors in the determination of this substance. It seems therefore that *in vitro* studies must be the deciding factor in determining whether cationic exchanges occur.

Turning now to the second class of work, a paper by Kerr may conveniently be chosen as the starting-point of this description.

Kerr (1929) placed cells of several species in salines containing varying amounts of sodium and potassium and after about 2 hours of suspension analysed the cells for any changes which may have occurred. Changes in the volume of the cells, which, as mentioned above, would have led to erroneous conclusions if not corrected for, were corrected for by multiplying the observed potassium or sodium content of the cells by the factor:

$$\frac{\% \text{ H}_2\text{O in test cells}}{\% \text{ H}_2\text{O in control cells}}$$

In actuality this was an incorrect factor and might lead to considerable errors. The correct factor may be found by considering the following example: Suppose we have 100 ml. of cells containing 70 gm. of water and 400 mg. of potassium. Suppose no change in potassium content occurs but that the cells contract in volume by 20% due to the loss of water. The observed potassium content becomes now 400 mg. per 80 ml., i.e.

$$400 \times 100/80 = 500 \text{ mg. per 100 ml.}$$

To bring this observed value to the true one, it must be multiplied by the factor 80/100, i.e. the ratio of the volume of the cells in the two states. Suppose now we use Kerr's factor, the ratio of the water in the two cases. We have in the first case water = 70. In the second case $100(70-20)/80 = 50$. Multiplying, we get

$$\frac{400 \times 100 \times 5000}{80 \times 80 \times 70} = 446 \text{ mg. K per 100 ml.}$$

This factor is obviously incorrect, since it gives a value of 446 mg. K per 100 ml., whereas the corrected value should be 400 mg. per 100 ml.

In spite of this mistake, Kerr's experiments indicate a definite permeability of the erythrocyte to cations if the suspension medium is changed sufficiently.

Kerr also observed that the cells showed a greater permeability to cations if they were suspended in serum than in an isotonic saline solution. Thus ox erythrocytes suspended in a saline containing 200 mg. K per 100 ml. showed a greater increase in their potassium contents than did cells suspended in serum of which the potassium content had been raised to 200 mg. per 100 ml. This phenomenon was investigated by Davson (1934), with special reference to the leakage of potassium from ox cells suspended in isotonic NaCl solution.

It was shown that calcium was not an important factor in the leakage of potassium from the ox cell in saline solution; however, the interesting fact emerged that ox cells leak potassium into their own serum nearly as rapidly as they do into saline. This point was further investigated by Davson & Danielli (1938), whose results are shown in Table XXVII, and it may be seen that an important factor in this apparent leakage is the centrifuging of the cell suspension; this is particularly well shown in the case of dogfish erythrocytes, which may be separated from their serum without centrifuging. When this is done no evidence of an escape of potassium in serum is found, but when the cells are centrifuged a loss of up to 9% is found. In the case of the ox there is undoubted evidence of a continuous leakage of potassium, but this may be accentuated by centrifuging the cells more than once. The losses obtained by repeated washing of ox erythrocytes are interesting in that a limit to the amount of potassium lost is reached after the

washing. It may be that there are some cells which lose their potassium when treated in this way, whilst the remainder retain their impermeability. Recent work on the storage of human blood for long periods (up to one month) indicates a definite and

TABLE XXVII. THE EFFECT OF CENTRIFUGING AND RESUSPENSION ON LOSS OF K^+ FROM RED CELLS (DAVSON & DANIELLI, 1938)

Exp. no.	Species	Treatment	K^+ content
1	Dogfish	Cells allowed to settle once from serum	100
		Cells allowed to settle twice from serum	100
		Cells allowed to settle once from Ringer	100
		Cells allowed to settle thrice from Ringer	100
		Cells centrifuged once from serum	93
		Cells centrifuged thrice from serum	91
		Cells centrifuged once from Ringer	96
		Cells centrifuged thrice from Ringer	88
2	Ox	Cells centrifuged immediately	100
		Cells centrifuged after 3.5 hours	100
		Cells centrifuged after 19 hours	98
		Cells centrifuged after 24 hours	96
		Cells centrifuged after 48 hours	96
		Cells centrifuged after 68 hours	95
		Cells allowed to settle from saline	95.5
		Cells centrifuged from saline	88.5
3 a)	Ox	Cells centrifuged from serum once	100
		Cells centrifuged from serum twice	94
3 b)	Ox	Cells centrifuged from serum once	100
		Cells centrifuged from serum twice	97
3 c)	Ox	Cells centrifuged from serum once	100
		Cells centrifuged from serum twice	99
4	Horse	Cells allowed to settle from serum	100
		Cells centrifuged from serum once	100
		Cells centrifuged from serum twice	100
		Cells centrifuged from serum thrice	100
5	Ox	Cells centrifuged from serum	100
		Cells washed once in isotonic NaCl	79
		Cells washed twice in isotonic NaCl	72
		Cells washed three, four and five times	72
6	Rabbit	Cells allowed to settle from serum	100
		Cells centrifuged from serum	100
		Cells washed once in isotonic NaCl	98.5
		Cells washed twice in isotonic NaCl	95.5
		Cells washed thrice in isotonic NaCl	95.5

continuous leakage of potassium (De Gowin *et al.* 1940; Scudder, 1940). This may be restricted by addition of glucose (Smith, 1941; Maizels, personal communication), and addition of glucose or sucrose restricts haemolysis in stored blood by increasing the external osmotic pressure. The action of glucose however is probably partly due to its utilisation as a metabolite.

Davson & Danielli (1936) investigated the much quoted, of Mond (1927) to the effect that the impermeability of the erythrocyte to positive ions was due to the existence of a positively charged layer of protein in the erythrocyte membrane with an isoelectric point of pH 8.3. Mond described experiments purporting to prove that when the membrane was made more alkaline than pH 8.3, i.e. when the protein in the membrane became negatively charged, the erythrocyte became a selectively cation permeable cell. In other words, Mond was of the opinion that the erythrocyte membrane could behave as the amphoteric membranes, which change their selectivity to ions according to which side of their isoelectric point they are on. Careful study of Mond's paper reveals, however, the inadequacy of his experimental procedure, the main objection being that he brought the cells to the required pH without the use of buffers, so that in actuality when the cells were to be brought to pH 10.1, they were initially placed in a solution of pH 12.8, a pH which is destructive to tissues in general.

TABLE XXVIII. EFFECT OF VARYING THE pH OF THE MEDIUM ON THE K^+ AND Cl^- CONTENT OF OX ERYTHROCYTES (DAVSON & DANIELLI)

Exp.	Washing fluid		K^+	Cl^-
			mg. per 100 ml. corp.	mg. per 100 ml. corp.
1	Unwashed	7.4	64.8	.
	Ringer	7.4	56.0	.
	Ringer	9.0	56.0	.
		7.4	67.6	.
	Ringer + KCl	7.4	88.0	.
	Borate Ringer + KCl	8.3	88.0	.
		7.4	72.7	234
	Glycine Ringer	7.35	64.9	242
	Glycine Ringer	9.10	65.0	153
	Glycine Ringer	10.0	67.6	110
	Serum + Glycine Ringer 1:1	9.63	67.2	

Mond was also working in a medium which, apart from the added alkali, was electrolyte free; this of itself is sufficient to cause the ox erythrocyte to become permeable to potassium (Joel, 1915; Davson, 1939a). Working under properly controlled conditions, Davson & Danielli showed that Mond's claim was entirely un- (Table XXVIII). These authors have discussed the

possible effects of potentials on ionic permeability in a biological system and point out that it is the so-called thermodynamic potential across the membrane as opposed to the electrokinetic potential which is of most interest in this connection, and that the sign of the former potential is not necessarily changed by a change of the reaction of the medium (see Chapter XXI).

Ponder & Saslow (1930, 1931) and Ponder & Robinson (1934, 1936) have found that the erythrocyte of the rabbit shows anomalies in regard to the degree to which it will swell or shrink in hypotonic and hypertonic solutions; these authors claimed that the cause of these anomalies was the escape or penetration of cations due, presumably, to the deformation of the membrane by the changes of tonicity. If such a permeability could be induced, the results would certainly be interesting; however, Davson (1936) showed that although these anomalies in the swelling and shrinking of the cells could be confirmed they were not to be accounted for on the basis of a permeability to cations. Nevertheless, Jacobs *et al.* (1936) claimed, also on the basis of indirect studies, that in strongly hypotonic solutions erythrocytes became permeable to potassium provided the temperature was maintained at about 40° C. A stretching of the erythrocyte membrane so as to enlarge pores which would allow of the escape of potassium is by no means an unreasonable assumption, and it was considered quite possible that the failure to observe measurable escapes of potassium from the rabbit erythrocyte in hypotonic solutions was due to the lower temperature at which the work was carried out (20° C.); the matter was therefore reinvestigated using six different species of erythrocytes (Davson, 1937). In Table XXIX some results are shown and it is clear that the permeability to cations claimed by Jacobs *et al.* at 40° C. is quite real and varies in degree according to the species chosen; in Fig. 34 curves describing the time course of the escape of potassium are shown; it is interesting that the permeability ceases when a certain loss of potassium has occurred, and this has been shown to be due to the shrinking of the cell to its normal volume owing to the loss of salt; thus a reversible permeability to cations may be induced in the erythrocyte by an apparently mechanical alteration of its membrane; the permeability is so small at 20° C. (the Q_{10} is approximately 2) that significant losses of potassium are difficult to detect. It is interesting to note that the permeability produced in this way is

TABLE XXIX. CHANGES IN THE POTASSIUM CONTENT OF ERYTHROCYTES OF DIFFERENT SPECIES ON EXPOSURE FOR 1 HOUR TO HYPOTONIC SALINE SOLUTION AT 5° AND 40° C. DAVSON, 1937

The potassium content of the untreated cells is put equal to 100. The concentration of the hypotonic saline is given in parentheses under each species. The values for the potassium content varied between 420 and 460 mg. per 100 ml. for the rabbit, horse, guinea-pig and pig; the human was rather lower at ca. 400 mg. per 100 ml. The values for the ox were of the order of 80 mg. per 100 ml.

Rabbit 0.10 <i>M</i>		Horse 0.12 <i>M</i>	
Temperature	100	100	100
5	99	99	98
40	86	89	87
Loss	13	10	11
Mean loss	11		
Ox 0.12 <i>M</i>		Guinea-pig 0.10 <i>M</i>	
Temperature	100	100	100
5	94	92.5	95
40	87	84.5	86
Loss	7	8	9
Mean loss	8		
Pig 0.12 <i>M</i>		Human 0.10 <i>M</i>	
Temperature	100	100	100
5	98	98	98.5
40	94.5	94	95.5
Loss	3.5	4	3
Mean loss	3.5		

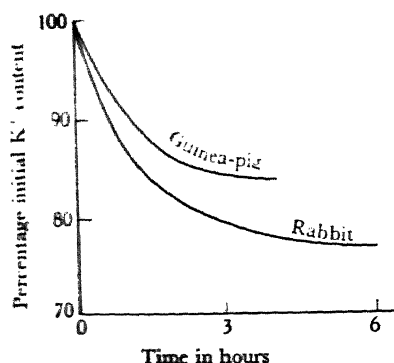


FIG. 34. The time course of loss of K^+ from red cells in hypotonic (0.1 *M*) saline. The saline, though hypotonic, is not sufficiently so to be haemolytic.

by no means large enough to account for the observations of Jacobs *et al.* (1936), which led them to postulate such a permeability, and it seems therefore that indirect studies, such as those on fragility, on the erythrocyte may give illusory results when translated into terms of ionic permeability, and until more is known about the equilibrium conditions pertaining to the erythrocyte such results must be confirmed by direct chemical methods.

Hypotony is a condition which may be described as pre-haemolytic, and it is consequently of interest to see whether other pre-haemolytic conditions were also accompanied by a permeability to cations. Ørskov (1935) has shown that the erythrocytes of several species become permeable to potassium on treatment with the heavy metal lead, a substance which in higher concentrations causes haemolysis, and a systematic study of a variety of haemolytic agents by Davson & Danielli (1938) indicates that, with the exception of saponin, any change in the environment which will cause haemolysis, if carried beyond a certain degree, will at the same time cause a leakage of potassium from the otherwise intact erythrocytes. These facts are demonstrated in Table XXX; it is interesting to note that the stronger the haemolytic agent used the smaller is the escape of potassium. Furthermore, the effect of a heavy metal like silver is a reversible one. In the same paper Davson & Danielli take up the problem of the high concentration of potassium in the erythrocyte and show that it is apparently not maintained by a metabolic process which is normally inhibited by such agents as cyanide, carbon monoxide or urethane, or accelerated by methylene blue or pyocyanine, and consequently it was considered that the high concentration gradient must be considered as a static phenomenon, in probable contrast to similar gradients in plant cells.

Amongst the metabolic poisons studied was fluoride, which gave results which agreed with those obtained by use of the other substances. However, these studies were made at 25° and Wilbrandt (1937) showed that fluoride will cause a rapid escape of potassium from the erythrocyte at 38° , an escape which he was inclined to believe was due to the poisoning of a metabolic process which maintained the high potassium concentration gradient. Davson (1941) has been able to confirm this claim, but has brought out two especial features of the process which would strongly indicate that this simple view is incorrect. The

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TABLE XXX. THE EFFECT OF LYSINS ON THE K^+ CONTENT OF RABBIT ERYTHROCYTES EXPRESSED AS A PERCENTAGE OF THE CONTROL VALUES

Corrections have been made for haemolysis so that any value less than 100 indicates an escape of K^+ . Time 1 hour. Temperature 25° C. The concentrations of lysozyme are expressed as fractions of a saturated solution: e.g. $2 \times 10^{-3} = \frac{1}{50}$ saturated solution

Lyso	Concentration	% haemolysis	Cell K content
Saponin	1 in 10,000	7	100
	0.9 in 10,000	4.5	100
	0.8 in 10,000	2	100
	0.7, 0.6, 0.5 in 10,000	None	100
Digitonin	4×5	7.5	97
	5×5	5	96
	3×5	3	99
	2×5	1	101
	$\times 5$ and $\times 10$	None	100
Na oleate	2.0 in 10,000	10.5	93.5
	1.8 in 10,000	8	95
	1.6 in 10,000	5	97
	1.4 in 10,000	3	97
	1.0 in 10,000	2	100
	0.5 in 10,000	1	100
Na valerate	0.2, 0.3, 0.4, 0	None	100
Na ricinate	4.0 in 1000	10	96.5
	1.6 in 1000	5	100
	1.0, 0.8, 0.8, 0.7 and 0.6 in 1000	None	100
Quinol	0.075 <i>M</i>	ca. 5	27.5
	0.050 <i>M</i>	ca. 5	41
	0.025 and 0.01 <i>M</i>	None	100
Resorcinol	0.163 <i>M</i>	None	16.9
	0.145 <i>M</i>	None	33.8
	0.131 <i>M</i>	None	62.0
	0.091 and 0.036 <i>M</i>	None	100
Catechol	0.136 <i>M</i>	ca. 5	9.1
	0.091 <i>M</i>	ca. 5	51.5
<i>o</i> -AmOH	0.18 <i>M</i>	None	87
	0.09 <i>M</i>	None	97
	0.07 <i>M</i>	None	100
Guaiacol	0.04 <i>M</i>	None	84
	0.03 <i>M</i>	None	92
	0.02 <i>M</i>	None	96.5
	0.01 <i>M</i>	None	99
Guaiacol (dogfish)	0.01 <i>M</i> (pH 5)	None	85
	0.01 <i>M</i> (pH 6)	None	86
	0.01 <i>M</i> (pH 7)	None	83
	0.01 <i>M</i> (pH 8)	None	83
	0.01 <i>M</i> (pH 9)	None	85
	0.01 <i>M</i> (pH 10)	3	70

first point is concerned with the effect of concentration of fluoride; Fig. 35 shows that the effect may fall off considerably by increasing the concentration above a certain value, so that in 0.165 *M* NaF the K escape is negligible; since glycolysis is inhibited at all the concentrations above the optimal one shown in the curve, it is

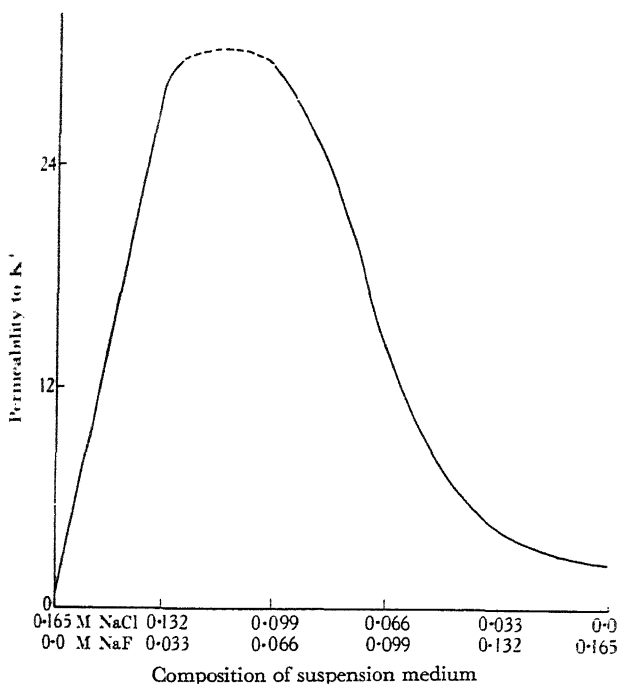


FIG. 35. Effect of varying the proportion of 0.165 *M* NaCl and 0.165 *M* NaF on the escape of potassium, P_K , expressed as a percentage loss per hour from the rabbit erythrocyte at 39.5° C. (Davson, 1941).

clear that inhibition of glycolysis alone is not the complete story. The second fact refers to the effect of a preliminary washing of the cells; in this case the fluoride effect is considerably delayed, so that whereas with unwashed cells a loss of 75 % of the potassium is found in 2 hours, with washed cells the loss is only 17 % in the same period. Washing the cells is more likely to depress glycolysis and therefore increase the fluoride effect (if the latter is merely to inhibit a secretory accumulation of K^+) than the reverse, so that

some other explanation is necessary. The most satisfactory explanation is that the presence of fluoride causes the accumulation of intermediate metabolic products which react with the membrane causing potassium permeability—a view which is also held by Wilbrandt (personal communication). It is known that high concentrations of fluoride tend to inhibit many stages of glycolysis. Inhibition spreads to the left and thus it is possible that, with the high concentrations, even the intermediate products whose accumulation causes the change of permeability will not be needed. The effect of washing the cells can be explained as a result of this; this is confirmed by the observation that adding serum, an ultrafiltrate of the same or an artificial Ringer solution containing Na, K, Ca, Mg, phosphate, chloride, bicarbonate and glucose in the correct proportions, causes the escape of potassium to occur as rapidly as if the cells had not been washed. The effects of Ringer solutions in which one component is removed are instructive and shown in Table XXXI; it is clear from the table that all the components are important, but that by far the

TABLE XXXI. THE EFFECT OF VARYING THE NATURE OF THE SUSPENSION MEDIUM ON THE ESCAPE OF POTASSIUM, P_K , FROM RABBIT ERYTHROCYTES IN 0.04 M FLUORIDE

Addition of 1 ml. of serum, Ringer solution and modified Ringer solutions were made to 10 ml. of a NaCl-NaF suspension medium. The cells were previously washed with NaCl solution. Units of P_K are percentage loss per hour. Temperature 39.5°C.

Suspension medium	P_K
NaCl-NaF	10
.. + serum	33
.. + Ringer	29
.. + Ringer—glucose	23
.. + Ringer—phosphate	26
.. + Ringer—calcium	19
.. + Ringer—magnesium	10
.. + Ringer—potassium	28

More recent work of Dean (personal communication) with radio-active isotopes strongly suggests that *in vivo* the erythrocyte has a small permeability to potassium, corresponding perhaps to a loss of 1% per hour; if this is the case, it is certainly necessary to invoke a secretory process which either accumulates potassium or pumps out sodium. Some recent results from De Gown's laboratory (personal communication from Dr Smith) on the escape of potassium during storage of human blood also

that this escape is not a pre-haemolytic escape in the sense of Davson & Danielli, but is rather due to failure of glycolysis to maintain the high potassium concentration in the cells. Whatever may be the true story regarding the maintenance of the high level of potassium in most species of erythrocyte, it is clear that the problem of the extremely low permeability (impermeability if there is no metabolic process) of the membrane to potassium in comparison with the high permeability to negative ions is a real one and must be sought in an analysis of membrane structure.

The escape of potassium from erythrocytes treated with light in the presence of rose bengal, described by Davson & Ponder (1940), presents interesting features which are best discussed under the heading of haemolysis, but it may be mentioned here as another instance of a pre-haemolytic cation permeability.

The behaviour of the erythrocyte in non-electrolyte solutions has both a practical and a theoretical interest; practical since many permeability studies are carried out in a virtually electrolyte free medium as, e.g., in the "haemolysis technique", and it is therefore interesting to see to what extent a cell in such a medium can be considered normal; and theoretical since an erythrocyte in a non-electrolyte medium will have considerable potential gradients across its membrane due to unequal distributions of ions (Chapter xv). Joel (1915) has shown that if the erythrocytes of the ox are washed repeatedly with sugar solution and then suspended in the same medium, the latter shows an increase in electrical conductivity with time which is due, as he supposed, to the passage of salts from the cells. Jacobs & Parpart (1933), on the basis of fragility studies, came to similar conclusions and Maizels, by direct chemical methods, showed that human erythrocytes definitely lose potassium when suspended in a non-electrolyte medium. Davson (1939*a*) has investigated the problem systematically, comparing the behaviour of seven different

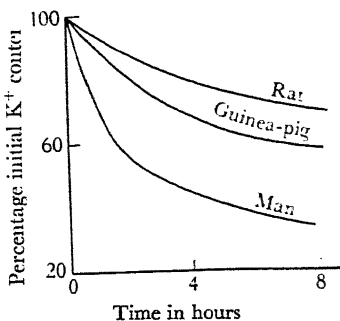


FIG. 36. The loss of K^+ from red cells when blood is diluted with 10 volumes of isotonic sugar solution at $25^\circ C$.

In Fig. 36 the loss of potassium with time is shown for the

erythrocytes of the rat, guinea-pig and man when the blood is diluted with ten times its volume of isotonic sugar solution: it is to be noted that the rate of loss falls off with time in a manner which cannot be accounted for on the basis of the kinetics of a single permeability process: it is concluded that the migration of potassium from the cells is accompanied by sufficiently large pH changes to cause potential differences opposing the further escape, thereby bringing the permeability to an end. It was found that the changes produced in the membrane by the non-electrolyte medium are quite reversible, so that addition of electrolytes to

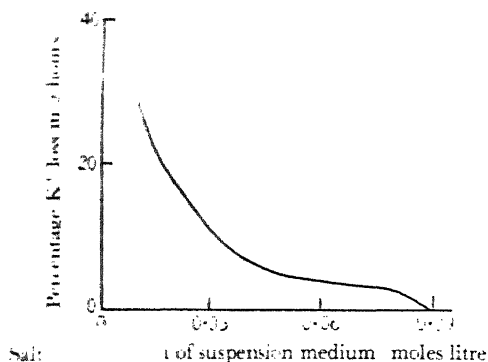


FIG. 37. Effect of salt concentration on the rate of loss of K^+ from human red cells with variation in the salt content of the suspension medium, at $25^\circ C$.

suspension medium causes an immediate cessation of the dialysis process. Other points are that the rate of escape is increased by alkalinity and decreased by acidity (see also Maizels, 1935) and that the escape of potassium becomes detectable when the salt concentration has been reduced below about $0.09 M$, i.e. when it is greater than half the isotonic concentration. When the dilution of the blood with isotonic non-electrolyte is really great (in permeability studies the dilution is often 1:500, i.e. the concentration of salts is about $0.0002 M$), the rate of escape of potassium may be many times that found in the experiments of Maizels (1935), where a dilution of 1:10 was used giving a rate of about 25% loss in the first 2 hours (for human cells). The amount of a penetrating non-electrolyte which must enter the cells to cause a significant loss of potassium is leaking from the cells will be greater

than if the cell retained its normal impermeability to cations; also the experiments indicate a definite abnormality in the erythrocyte when in a non-electrolyte medium.

In this description of cationic permeability in the erythrocyte we have dealt with what appear to be abnormal permeabilities, the assumption being that in its normal environment the erythrocyte studied was impermeable to potassium; hence this work might more aptly be described as studies of the conditions determining the normal impermeability of the erythrocyte membrane to cations. We have mentioned earlier that *in vivo* experiments with certain species, notably the cat and dog, indicate a normal permeability to cations, either sodium or potassium or both, and it would be of great interest to enter into some of the characteristics of this form of permeability, e.g. the influence of calcium, pH, narcotics, etc. The only systematic study of this form of permeability in the erythrocyte is that of Davson (1939*b*, 1940*a, b*), using the cat erythrocyte as the experimental object. The cat erythrocyte contains mainly sodium (Table XXXVI), so that if it is suspended in isotonic potassium chloride solution there will be a concentration difference of sodium in the direction cells—suspension medium of magnitude about 0.16 mole per litre and a concentration difference of potassium of about the same magnitude in the reverse direction. Hence, if the cells are permeable to sodium and potassium, under these conditions the former will leave the cell and the latter will enter it. In Fig. 38 changes in the sodium and potassium concentrations of the cat erythrocyte with time of suspension in isotonic potassium-chloride solution have been plotted. Since the concentration differences of sodium and potassium are about equal, the slopes of the curves indicate the relative permeabilities of the membrane to sodium and potassium, and it is to be noted that sodium diffuses through about five times faster than potassium; this result is unexpected since, if ionic radii determine the rate of penetration, the reverse should be true. Davson has investigated whether the permeabilities observed are artificial in that they are due to dilution of the plasma or to the presence of a high concentration of potassium. It was found that dilution of the plasma is not a factor of importance; however, the concentration of potassium is of importance in regard to sodium permeability, as Fig. 39 shows, where the rate of penetration of sodium expressed as a percentage decrease

PERMEABILITY OF ERYTHROCYTES TO IONS

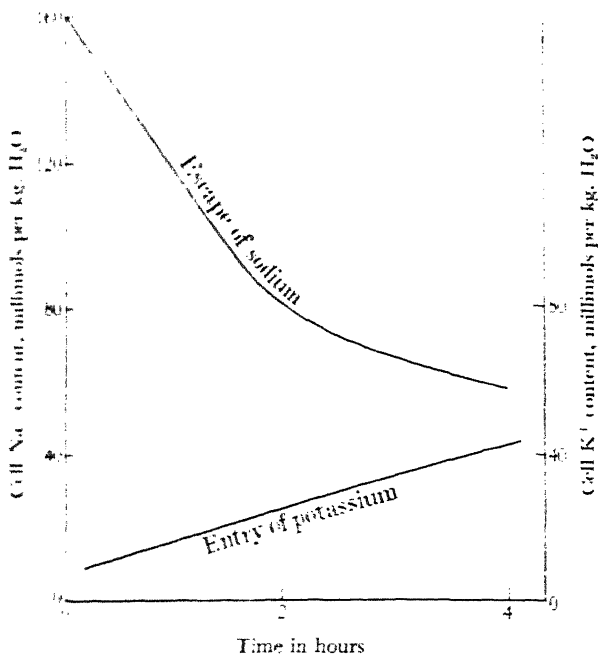


FIG. 38 Changes in the Na⁺ and K⁺ in cat red cells when suspended in isotonic KCl solution at 25° C.

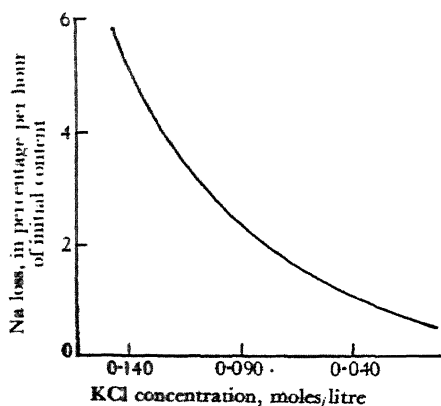


FIG. 39 Effect of variation in the external K⁺ concentration on the rate of leakage of Na⁺ from the cat red cell.

per hour is plotted against the concentration of potassium in the suspension medium, the tonicity being maintained constant by replacing the isotonic potassium chloride with isotonic sodium chloride. If the concentration of potassium is without influence, the plot should be a straight line; in actuality it is seen that increasing the concentration of potassium, or more correctly the proportion of potassium to sodium, in the medium causes an increase in the rate of penetration greater than that demanded by the increased concentration difference of sodium.

We have seen that hypotonicity may influence the normal impermeability of the erythrocytes of some species (p. 146); is the permeability of the cat erythrocyte to potassium and sodium influenced also? Table XXXII shows that the membrane

TABLE XXXII. THE EFFECT OF VARYING THE TONICITY OF THE MEDIUM ON THE RATE OF PENETRATION OF POTASSIUM, P_K , AND THE RATE OF ESCAPE OF SODIUM, P_{Na} , EXPRESSED AS PERCENTAGE INCREASE OR DECREASE OF THE INITIAL CONTENT PER HOUR (DAVSON, 1940a)

(Corrections have been made for changes in the concentration differences due to variations in the amounts of potassium and sodium in the suspension media and due to shrinkage or swelling of the cells)

Composition of suspension medium	P_K	P_{Na}
0.225 <i>M</i> KCl	.	23.5
0.205 <i>M</i> KCl	.	24.5
0.185 <i>M</i> KCl	120	20.0
0.175 <i>M</i> KCl	122	16.0
0.165 <i>M</i> KCl	128	8.5
0.150 <i>M</i> KCl	138	3.0
0.140 <i>M</i> KCl	143	0
0.130 <i>M</i> KCl	150	0
0.110 <i>M</i> KCl	185	.
0.165 <i>M</i> KCl + 0.06 <i>M</i> NaCl	.	14
0.165 <i>M</i> KCl + 0.04 <i>M</i> NaCl	106	16
0.165 <i>M</i> KCl + 0.02 <i>M</i> NaCl	109	16.5
0.165 <i>M</i> KCl + 0.01 <i>M</i> NaCl	115	15.5

responds in two different ways according as sodium or potassium permeability is being studied, hypotonicity causing an acceleration of the latter and an inhibition of the former; hypertonicity, on the other hand, has the reverse effect in each case. This difference in response to an environmental change is striking and unexpected, and it is of interest to see whether any other changes in the erythrocyte's environment will produce contrary effects. In Table XXXIII is shown the effect of various concentrations of the alkali earths on the rate of penetration of potassium and

sodium through the membrane of cells suspended in isotonic KCl to which the alkali earths have been added. The table shows that in concentrations of 0.01 *M* and over, calcium *accelerates* the rate of penetration of potassium: in lower concentrations it has no effect; magnesium showed an irregular effect, sometimes

TABLE XXXIII. THE EFFECT OF ALKALI EARTHS ON THE RATE OF PENETRATION OF POTASSIUM INTO P_K AND THE RATE OF ESCAPE OF SODIUM FROM P_{Na} OF THE ERYTHROCYTES OF THE CAT (DAVSON.)

P represents the percentage change in the potassium or sodium contents of the erythrocytes per hour; the values have been corrected for changes in the concentration difference due to variations in tonicity

Suspension medium		
0.165 <i>M</i> KCl	186	8
0.165 <i>M</i> KCl + 0.04 <i>M</i> Mg	186	21.5
0.165 <i>M</i> KCl + 0.02 <i>M</i> Mg	204	18
0.165 <i>M</i> KCl + 0.01 <i>M</i> Mg	199	15
0.165 <i>M</i> KCl + 0.005 <i>M</i> Mg		11.5
0.165 <i>M</i> KCl + 0.001 <i>M</i> Mg		10.5
0.165 <i>M</i> KCl		7
0.165 <i>M</i> KCl + 0.04 <i>M</i> Sr	155	13.5
0.165 <i>M</i> KCl + 0.02 <i>M</i> Sr	155	.
0.165 <i>M</i> KCl + 0.01 <i>M</i> Sr	153	9
0.165 <i>M</i> KCl + .	.	8
0.165 <i>M</i> KCl + .	165	
0.165 <i>M</i> KCl	118	10.5
0.165 <i>M</i> KCl + .	175	18.5
0.165 <i>M</i> KCl + 0.01 <i>M</i> Ca	124	15.5
0.165 <i>M</i> KCl + 0.005 <i>M</i> Ca	118	14
0.165 <i>M</i> KCl + 0.001 <i>M</i> Ca	118	10.5
0.165 <i>M</i> KCl	179	10
0.165 <i>M</i> KCl + 0.04 <i>M</i> Ba	133	19
0.165 <i>M</i> KCl + 0.02 <i>M</i> Ba	138	18.5
0.165 <i>M</i> KCl + 0.01 <i>M</i> Ba	157	16
0.165 <i>M</i> KCl + 0.005 <i>M</i> Ba	167	13.5
0.165 <i>M</i> KCl + 0.001 <i>M</i> Ba	184	11

producing a slight acceleration and sometimes the reverse—the changes were so small as to lie very nearly within the limits of error. Strontium and barium retarded potassium permeability at all the concentrations studied. It was found in more detailed investigation that the action of calcium is dependent on the Na : K ratio of the medium, a high ratio causes calcium to inhibit potassium permeability whilst a low ratio causes only acceleration or has no influence depending on the calcium concentration. Hence, in this case, we may speak of an antagonism between sodium on the one hand and potassium and calcium on the other.

The general rule of Loeb, to the effect that bivalent salts are antagonistic to monovalent ones, is not sustained by these experiments, since calcium and potassium acting together produce an additive effect.

Turning now to the effect of the earths on sodium permeability we note, once again, a remarkable difference in response of the membrane to its changed ionic environment according to which cation is being studied. Thus whilst barium and strontium invariably decrease the permeability of the membrane to potassium the table shows that these ions invariably accelerate the rate of loss of sodium; again, the action of magnesium on potassium permeability was negligibly small, whilst this earth causes a well-defined acceleration of sodium permeability; similarly, whilst calcium only accelerates potassium permeability at concentrations greater than $0.01 M$ and has no influence on it at lower concentrations, the action of this earth on sodium permeability is acceleratory at all the concentrations studied above $0.001 M$. We have already seen that hypertonicity causes an acceleration of sodium permeability, so that it is clear that a part of the acceleration produced by the added alkali earths will be due to this cause, especially in the higher concentrations: however, further investigation of this point has shown that the tonicity changes are responsible for only a part of the observed effects of the alkali earths; the essential point to be noted, so far, is the obvious difference in response of the membrane to added alkali earths according as sodium or potassium permeability is being considered.

The Effects of Age on Permeability. In the work described so far, the blood was generally drawn on the evening previous to its being used, so that it had stood for 12 hours or so in the ice-chest; this was adopted as a routine procedure for convenience only. However, on occasions it was found that the rate of escape of sodium from the erythrocytes suspended in isotonic KCl solution was very much greater than that usually found (20–30% per hour against 8–10% per hour usually found). Eventually the variable was traced to the use of freshly drawn blood. In Fig. 40 the losses of sodium from fresh and one-day-old blood are plotted against time of suspension in isotonic KCl. The rates of escape of sodium from the same blood when freshly drawn and on the first, second and third days following withdrawal were as follows: 26,

17.5, 11.5, 11.5 similar figures for the rates of penetration of K^+ 100, 104, 104. The figures represent the percentage loss of sodium or gain of potassium in 1 hour. It is evident that the changes in the membrane cease by the third day. This effect of age on permeability of the cat erythrocyte is surprising, as one would naturally expect the opposite relationship, the older cell being more "leaky" than the new one. It is to be noted that the effect of age on permeability to potassium is less

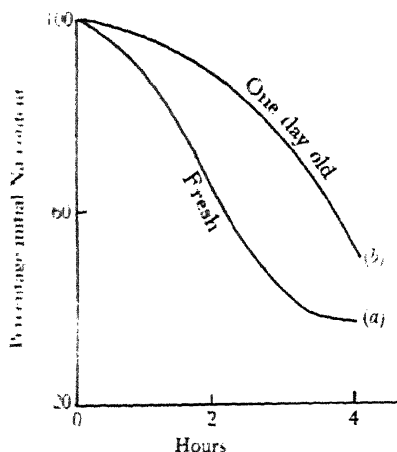


Fig. 1. Loss of Na^+ from cat red cells in isotonic KCl : (a) when fresh; (b) after keeping in serum for 24 hours.

with sodium permeability; hence once again we find a real distinction in the behaviour of the red cell in respect to sodium and potassium permeability.

The Effect of pH on Permeability. In Table XXXIV is shown the effect of varying the pH of the suspension medium on the rate of penetration of potassium and the rate of loss of sodium from the erythrocyte; phosphate- KCl mixtures were used to produce the pH changes. It is seen that in this case the effect of pH is qualitatively the same for both sodium and potassium permeability; quantitatively the effect of pH is much more marked in respect to sodium permeability. Thus the increase in the rate of penetration of sodium, on passing from pH 6 to pH 7.9, is nearly fivefold, whereas for potassium permeability it is less than twice.

TABLE XXXIV. THE EFFECT OF THE pH OF THE SUSPENSION MEDIUM ON THE PERMEABILITY OF CAT ERYTHROCYTES TO P_K AND SODIUM P_{Na} (DAVSON, 1940a)

7.9	5.6	6.6	6.0	5.8
305	268	220	190	
25	26	10.5	5.5	2.0

The Effect of Narcotics. It is generally believed [see Chapter XVI] that narcotics decrease permeability in low concentrations and in higher concentrations accelerate it, the latter phenomenon often being irreversible.

The effect of *n*-butyl carbamate, a typical narcotic, on the permeability of the cat erythrocyte to sodium and potassium has been studied with cells suspended in isotonic potassium-chloride solution. Once again we are confronted with an astonishing difference in behaviour of the erythrocyte in respect to potassium and sodium permeability; in the former case only an acceleration of the rate at high concentrations is observed with no inhibition of the rate at all at the lowest concentrations. With regard to sodium permeability we find a well-marked retarding action of the narcotic amounting to a complete inhibition of sodium escape at concentrations above 0.3% *n*-butyl carbamate. This difference in behaviour of the cells with regard to their permeability to sodium and potassium becomes still more striking when one notes that the same concentration of narcotic, which inhibits completely the permeability of the membrane to sodium, accelerates the rate of penetration of potassium under precisely the same conditions. In Chapter XVI the effects of narcotics on sodium permeability will be described in greater detail.

The Effect of the Lyotropic Series of Anions on Permeability Phenomena. The literature contains the results of a great deal of work on the influence of the lyotropic series of anions on physiological phenomena. The idea which motivated these studies was that cells, including their membranes, constituted a colloidal system, and since the most characteristic feature of colloids was then believed to be the influence of hydration on their physical state, it was argued that if certain physiological phenomena showed influences by ions which normally affected the degree of hydration of colloidal systems, then the systems determining the physiological behaviour were in themselves colloidal. Thus Höber (1909) has studied the influence of anions on the ciliary movement in the

epithelium of the frog's stomach and has found that if the anions are arranged in the order of their activities a lyotropic series is obtained. Similarly, Port (1910) has studied the effect of anions on saponin haemolysis, Raber (1920) changes in the apparent permeability of *Lammaria*, Gellhorn (1920) changes in the motility of spermatozoa, and Loeb & Cattell (1915) the proportion of *Aurearia* embryos recovering from potassium poisoning, to name just a few of the many investigations of this nature. It is on the basis of work of this nature that Hober (1922) has developed the so-called "Colloid-chemical View-point" in regard to physiological phenomena: according to this view-point, the membrane of the cell is a colloidal system and its permeability is determined by its degree of hydration; dehydrating substances, such as sodium sulphate, were supposed to exert a thickening or packing effect on the micelles constituting the membrane and so decrease permeability; hydrating substances such as sodium thiocyanate posed, on the other hand, to loosen the membrane and thus increase permeability.

A careful investigation of the claim that sulphate decreases permeability and that thiocyanate increases it reveals that this is by no means a general rule: if we confine our attention only to the work quoted by Hober (1922) we find that Raber (1920) reports the reverse relationship, as do also in one instance Loeb & Cattell (1915). Again, the evidence put forward by Port (1910) on the effect of the series of anions on saponin haemolysis has not been confirmed by the more exact measurements of Ponder (1934), and Hober (1908) in his study of hypotonic haemolysis ignores the shrinking of the erythrocyte caused by the presence of a divalent ion such as sulphate through ionic exchanges. In view of these considerations it would appear that the factual basis for the colloid-chemical view-point is not strong, and it appeared of interest to one of us to investigate the influence of the series of anions on a comparatively simple permeability system.

The cat erythrocyte is a suitable subject for the study of the effect of the lyotropic series of anions on ionic permeability; thus if cells are suspended in isotonic solutions of KCNS, KCl, KI, KBr, potassium acetate, etc. and the rates at which potassium enters and sodium leaves the cells are measured, influences of the anions may be determined. However, Davson (1940 *b, c*) has pointed out that a series of anions may exert influences on cation

permeability merely in virtue of the different diffusion potentials created; thus the rate at which potassium will enter the cells will be measured by the factor $RT \frac{2l_c l_a}{l_c + l_a}$, where l_c refers to the mobility of the cation through the membrane and l_a that of the anion, and if l_a is different for different anions it is clear that the observed rate of penetration of potassium when cells are suspended in solutions of different potassium salts will vary in accordance with the mobility of the anion of the salt. Hence an influence of an anion on cation permeability is not necessarily to be attributed to an influence on the structure of the membrane, and it may be that this purely electrical effect was a factor in the many studies of the influence of the lyotropic series on physiological activity, since the activity of tissues and cells is bound up in some way not yet clearly understood with the migration of ions across a membrane.

If the quantity of potassium penetrating in 1 hour is used as an index to rate of penetration, the following series is obtained:

CNS>	I	>	NO ₃	>	Br	>	Cl	>	acetate	>	oxalate	>	SO ₄	>	citrate	>	tartrate
178	143	133	112	100	85	84	56	45	29								

i.e. the series is that known as the lyotropic series. However, we have seen earlier that the anions penetrate in the same order as the lyotropic series, so that the effects observed here may be due to the electrical effect discussed above. In Fig. 41 curves for the penetration of the potassium salts of a number of acids are given; the curves for chloride, nitrate, bromide and acetate lie very close together and the differences in their rates are not very consistent since they actually cross over; iodide, thiocyanate and cyanide show a very decided acceleration, however. Sulphate, tartrate and citrate show a very decided retarding effect as compared with chloride. Thus the influence of the lyotropic series of anions is qualitatively what would be expected on the basis of the diffusion potentials set up; i.e. qualitatively it would seem that the anions have no effect on the membrane itself.

As the permeability constant for potassium is of the order of 1×10^{-19} gm. mol per sq. micron per mol per litre concentration difference, and yet the Q_{10} is only about 2, it is probable that penetration must be through a small fraction of the surface area.

The effect of the anion series on sodium permeability is altogether different from its effect on potassium permeability: in Fig. 42 representative curves are shown and it is evident that the anions on the hydrating end of the series cause an almost complete inhibition of permeability. The effects of sulphate, tartrate and citrate would also appear to be inhibitory as compared with chloride, but this is probably due to the delay in penetrating the cell; thus if cells are suspended for 2 hours in Na_2SO_4 , so that

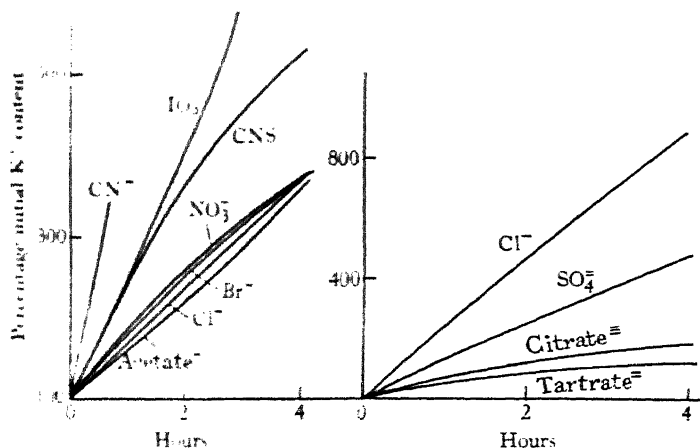


FIG. 41. Rate of penetration of various isotonic potassium salts into the cut red cell.

a complete exchange of the chloride and bicarbonate in the cells for sulphate has been made, then it is found that sodium escapes from the cells nearly twice as fast in K_2SO_4 as from cells added directly to KCl .

The full explanation of these effects awaits further experimental work: the extraordinary inhibiting effect of iodide and thiocyanate is strongly reminiscent of the action of narcotics mentioned earlier; these substances completely inhibit sodium permeability, leaving potassium permeability unchanged or accelerated. This narcotic action is not confined to those substances, ethers, alcohols, urethanes, etc., which are classified as narcotic substances, but is also shared with soaps, e.g. sodium oleate; hence it may be that

adsorbability at an oil-water interface besides lipid solubility is a factor in the inhibition of sodium permeability. The effect of the series of the monocarboxylic acids, acetic, propionic, etc. on sodium permeability is instructive and is shown in Fig. 43; it will be seen that the rates decrease slowly with increasing length of the hydrocarbon chain until we pass from valerianic to caproic when a complete inhibition of permeability is observed.

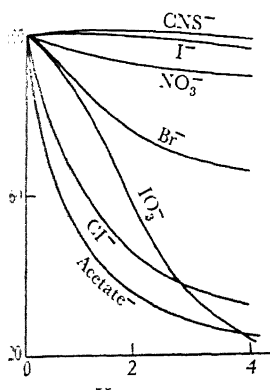


FIG. 42. Rate of leakage of Na^+ ions from the cat red cell into isotonic solutions of various potassium salts.

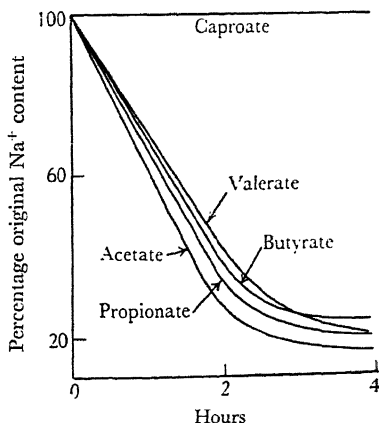


FIG. 43. Leakage of sodium from cat red cells in isotonic solutions of K^+ salts of monocarboxylic acids.

Whatever may be the cause of the effects of the lyotropic series of anions described by Davson it is quite clear that they are not simply to be ascribed to hydration influences on the membrane and that it is idle to build up a theory of membrane structure based largely on the observation of the influence of changes in the ionic environment of the medium on such complex physiological phenomena as ciliary movement, the activity of spermatozoa, resting potentials, etc.

We have presented the results of studies on ionic permeability in the erythrocyte in some detail because, although many of the results are not easily interpretable on the basis of membrane structure, they do represent *quantitative* studies, carried out by the most reliable of techniques, namely chemical, in the field of ionic permeability, a field which has been very little explored and,

where it has, often by far from satisfactory techniques. The experimental work on the erythrocyte may be considered to fall into two main groups in which the problems of ionic *impermeability* and ionic *permeability* are investigated. In the former class we have seen that a large variety of environmental influences—age, tonicity, lipid soluble substances, surface active substances, non-electrolyte media, photo-dynamic dyes, fluoride and temperature—are all effective under appropriate conditions in causing the erythrocyte membrane, which is apparently normally impermeable to potassium or at any rate virtually so, to allow the escape of potassium. A number of these influences can definitely be described as pre-haemolytic, i.e. they represent the initial changes in the erythrocyte membrane which, if allowed to continue far enough, will lead to haemolysis. These changes have been found in many instances, e.g. with heavy metals, and hypotonicity, to be reversible ones, so that the changes in the membrane are not extreme but probably represent some disorientation of its constituent lipid or protein molecules. The lipid soluble narcotics, e.g. amyl alcohol, which have this pre-haemolytic effect, may justly be expected to penetrate a surface lipid film and disturb the orientation of its molecules; the same may also be said for the soaps. The effect of a non-electrolyte medium will be discussed in Chapter XXI, but it may be stated now that a change in the packing of a surface film may certainly be expected by replacing the normal electrolyte medium surrounding it with non-electrolyte. The action of photo-dynamic dyes, e.g. rose bengal, is undoubtedly a chemical one and it is interesting to note that it is also irreversible and it must be assumed that small holes are actually made in the membrane by the oxidative process. The action of fluoride may also be regarded as a pre-haemolytic one, since it is observed that after 3–4 hours haemolysis appears and the permeability to sodium induced at the same time is not adequate to account for the degree of haemolysis observed; in this case the fluoride is pre-haemolytic as a result of the modification of the normal glycolytic processes occurring in the cell whereby an intermediate product is accumulated apparently in the cell or at its surface. Davson (1940) was inclined to include the effect of ageing as a pre-haemolytic change, but in the light of recent work by Smith (1941) it is possible that the small rates of loss observable are due to the

direct inhibition of an accumulatory process. Hypotonicity represents a stretching of the membrane and may be treated as a special case of pre-haemolysis; in this case the disturbance of the orientation of the membrane molecules is mechanical and possibly the effect of centrifuging the cells may also come under this category.

We have noted* that very strong haemolytic agents such as saponin produce a negligible pre-haemolytic potassium escape; Davson suggests that this may be explained on the assumption that saponin does not attack the membrane until it is present in the bulk phase in a suitable concentration and that it then reacts rapidly in an autocatalysed manner to produce a large hole in a very short time, so that, by the methods of measurement used, the escape of haemoglobin is synchronous with the potassium escape. It has also been noted that saponin, unlike other surface-active or lipoid-soluble substances investigated, has no narcotic action, and this would accord well with the assumption that below a certain bulk concentration saponin is without influence on the membrane, i.e. it does not penetrate it. The results on potassium impermeability in the erythrocyte make, therefore, a fairly consistent story whose general theme is that the impermeability is dependent on a correct orientation of the membrane molecules; this orientation may exert its effects partly spatially and also by the net electrical effects it produces.

From the second half of the experiments described in this chapter, namely those devoted to ionic *permeability* in the cat erythrocyte, few positive deductions may be drawn as yet since the system, which is probably simple in comparison with other biological ones, proves nevertheless to be very complicated in comparison with known physical ones. The comparison of sodium and potassium permeability suggests that the latter in the cat cell is more "normal" in the sense that the environmental influences, e.g. pH , tonicity, alkali earths, age, and anions, have effects with a magnitude and direction more in conformity with other permeability systems than do similar influences on sodium permeability. At the moment Davson (1940*c*) is inclined to draw a distinction of this sort between the two permeabilities and to class the sodium permeability with the "abnormal" permeabilities to glycerol which on other grounds have been shown to pertain in various species of erythrocytes. These two permeabilities, sodium

in the cat erythrocyte and glycerol in the rabbit for example, do show some remarkable similarities: e.g. an acid pH causes a sharp retardation of permeability in the rabbit-glycerol system; with the cat-sodium system, this may amount to a complete inhibition. Again, the rabbit-glycerol permeability is markedly reduced by narcotics, and strongly surface-active substances like soaps, but not by saponins, and similar results are found in the cat-sodium system; further, copper markedly inhibits the rabbit-glycerol permeability and, as we shall see in Chapter xvi, copper and other heavy metals have a similar inhibitory action on the cat-sodium system. If we note also the quite phenomenal effect of ageing in the cat-sodium system, and also the fact that P_{Na} is about five times P_K , we shall incline to the belief that the cat erythrocyte membrane is specially differentiated to permit the passage of sodium, and that this differentiated portion of the membrane has some of the characteristics of an enzyme* in its extreme susceptibility to pH , narcotic substances, heavy metals, age and perhaps to changes in orientation due to tonicity changes: it will be recalled that the effect of hypotonicity is quite sharply defined in that a normal permeability may be observed in $0.165M$ KCl and complete inhibition at $0.140M$. If this distinction between permeability processes is justified, we have in the cat-sodium system a form of permeability which may be regarded as an intermediate stage between the simple permeability process and the complicated secretory process which enables ions or molecules to move against concentration gradients. The sodium permeability is apparently not a secretory process, since sodium only diffuses to a state of lower chemical potential; nevertheless, the membrane would appear to be specially differentiated to deal with it in the same way that we must conceive of a specialisation of a secreting cell to certain secreted substances, e.g. the membrane of the kidney tubule cells in respect to chloride, phosphate, glucose, etc. Dean (*vide* Davson, 1940c) has suggested that the concentration of potassium in the erythrocytes of most species may occur by a pumping out of sodium instead of an accumulation of potassium; we have seen that the cat cell does not accumulate potassium (or pump out sodium) to any extent, so that the "abnormal" permeability to sodium may possibly be considered as a vestige

* This suggestion is discussed elsewhere in more detail in relation to glycerol penetration (Chapter viii).

of what was formerly a secretory process. Another phenomenon which has so far not been considered in relation to these results is that of anomalous osmosis (see Chapter xix), which Danielli 1942 points out must be a complicating factor in all cases where all pores existing in the cell membrane are not of the same size.

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CHAPTER XIII

PERMEABILITY TO IONS OF CELLS OTHER THAN THE ERYTHROCYTE

By H. DAVSON

TO describe adequately the phenomena of ionic permeability in cells other than the erythrocyte is difficult for the following reasons. First, consistent studies on any given cell or tissue by a single group of workers are rare, so that the information on a variety of cells is more or less of a casual nature. Secondly, chemical techniques have only been applied in extremely rare instances, so that the interpretation of results is too often equivocal. Thirdly, a large number of studies reputed to be permeability studies are in fact only remotely linked with the problem of permeability. Fourthly, the exchange of ions in most cells other than the erythrocyte is intimately associated with secretory processes, so that results uncomplicated by metabolic influences are rarely obtained. For these reasons the following discussion will appear fragmentary, and in many instances the choice of material to be presented will be more or less arbitrary.

Plant Cells. Reference has already been made to the experiments of Overton on plant cells in regard to the permeability of these cells to non-electrolytes. The method used by this author was the production of plasmolysis by a hypertonic solution; if the plasmolysis was permanent it was assumed that the cell was impermeable to the added non-electrolyte, whereas if de-plasmolysis occurred, permeability was presumed.

Overton (1895) used this method to study the permeability of plant cells to salts also, and concluded that in general plant cells are impermeable to salts since the plasmolysis was permanent. This claim was disputed by Osterhout (1911), who drew attention to the fact that plasmolysed *Spirogyra* cells will, under certain conditions, de-plasmolyse and then later plasmolyse again; this second plasmolysis, resulting presumably from damage to the cells, he characterised as pseudo-plasmolysis. The de-plasmolysis, which in the case of plasmolysis produced by pure NaCl solutions occurred within 10 to 30 min., was apparently due to the penetration of the sodium chloride. Fitting (1915, 1917, 1919) made

a careful study of plasmolysis with *Tradescantia* cells by salt solutions and confirmed Osterhout's observation of de-plasmolysis; he found, however, that the rate of de-plasmolysis slowed down with time, indicating, in his view, a decreasing permeability to salts; however Brooks (1916) has argued that Fitting's experiments were probably complicated by the exosmosis of salts from the de-plasmolysing cells. Fitting found that the rate of penetration of the salts presuming that the de-plasmolysis time is an index to this varied with the salt used and was in the order $K > Na > Li$ so far as cations were concerned and in the order $Br > NO_3 > Cl > ClO_3 > SO_4$ with regard to anions. The importance of the ionic milieu in respect to ionic permeability of plant cells was emphasised first by Osterhout (1911), who showed that *Spirgyra* cells will not plasmolyse in 0.375 *M* NaCl nor yet in 0.195 *M* $CaCl_2$, whereas in a mixture of 100 ml. of the NaCl solution and 10 ml. of the $CaCl_2$ solution plasmolysis will occur; in the first two instances presumably the membrane was too permeable to salts, whereas in the mixture the permeability was sufficiently low to allow of plasmolysis. Similar results on the importance of calcium have been put forward by Brooks (1916, 1917) with *Taraxacum* and *Laminaria* leaves, by Fitting with *Tradescantia* and by Kahho (1921) with lupin roots, and it would seem that pure solutions of sodium chloride increase the permeability to salts, this effect being antagonised by calcium chloride. Pure calcium chloride solutions, however, produce only a transient diminution in permeability.

A very detailed study of the influence of the ionic make-up of the medium surrounding the cell has been made by Osterhout (1922a) using indirect methods. This author pressed disks, cut from the fronds of a sea-weed *Laminaria*, between two conductivity electrodes and measured the electrical resistance at low frequencies when the disks were placed in sea water or other aqueous solutions. The assumption at the base of this work is that a significant proportion of the current is carried by ions which have to traverse the cell membranes as opposed to passing through the interstitial space. That this assumption is true has not been adequately investigated and it seems to the present authors that the comparatively low resistances found by Osterhout for intact cells would indicate that a large proportion of the current is being carried through the interstitial space. If we assume that this

interstitial space corresponds to about 15%, of the total cross-sectional area of a frond, it is clear that if the resistance of the intact cells is put equal to a hundred, the resistance of dead cells, i.e. cells which have become completely permeable to ions, will be about 15% of this value. Changes of this order between living and dead fronds have been found by Osterhout, so that it seems quite possible that little current was carried through the cells when intact. The possibility that this might be the case has been discussed by Osterhout (1922*b*); thus he finds that, like *Laminaria*, *Nitella* shows a decrease in resistance with time on suspension in NaCl, whereas if calcium is added this does not occur. By direct chemical tests on similar cells it was found that the nitrate ion in a properly balanced solution does not penetrate rapidly enough to give a qualitative test for its presence in less than 48 hours; without calcium the test was obtained in 3 hours. If the mobility of ions through the membrane is such that it requires more than 48 hours to bring about a concentration equilibrium, it is clear that its resistance must be many thousands of times greater than that of sea water, or than when it is dead; yet changes of this order have never been found by Osterhout.

The experiments of Osterhout, in spite of these objections, are very interesting in that they measure *increases* of permeability to ions; hence the process of death may be followed by electrical conductivity methods. It is found that in pure NaCl solution the resistance increases continuously with time; this increase is, within certain limits, reversible in that replacement of the NaCl solution with sea water brings it back to its original value. The increase in resistance may be inhibited by addition of calcium. Pure solutions of alkaline earths and lanthanum nitrate caused an initial rise in resistance followed, however, by a rapid and irreversible fall; the rise in resistance is hard to interpret but may be connected with changes in the volume of interstitial space.

The same technique has been applied by Raber (1920), who finds that the rate of increase of conductivity ("permeability") in pure solutions of sodium salts varies with the anion, being greatest in citrate, phosphate and tartrate solutions and least in the halides. A great objection to this technique apart from the criticisms already raised is, of course, that the investigator is quite ignorant of the ion or ions whose "permeability" changes are measured; thus if a rise in resistance occurs on placing *Laminaria*

in pure lanthanum nitrate solution as compared with sea water this may be due to a change in mobility of the sodium, potassium, chloride, lanthanum or nitrate ions which will all be present in such a system, the first three being inside the cells, the others mainly outside.

Marine Algae. These plant cells are ideal for the study of plant permeability, since those of certain species are large enough for the sap to be removed from an individual cell and analysed chemically. In Table XXXIV are shown the chloride, sodium, potassium, calcium, magnesium, sulphate, phosphate and nitrate contents of the saps of *Valonia macrophysa*, *Valonia ventricosa*, *Halysotis Osterhoutii*, *Nitella clavata* and *Chara ceratophylla*, and of the waters by which they are normally bathed. The table is taken from Osterhout 1936. The table shows that *Chara*, *Valonia* and *Nitella* all contain a very much higher concentration of potassium than that in their normal medium, and most of the experimental studies of these plant cells have been concerned with the mechanism of the accumulation of this ion. The figures for the internal pH of the cells included in Table XXXV show that the reaction is considerably more acid than sea water and hence there is the possibility that the high concentration of potassium represents an equilibrium state similar to that described in muscle (see Chapter III: Osterhout and his collaborators in a series of papers too numerous to be quoted individually here (for reviews see Osterhout, 1933, 1936) assume that in *Valonia* the high concentration of potassium represents a "steady state" brought about by the continuous formation of organic acid metabolites in the cell, so that the condition $K_i \times OH_i = K_o \times OH_o$ (where the suffixes *i* and *o* refer to the inside and outside of the cell respectively) is satisfied; if the internal pH falls due to the formation of excess of acids, then there will be a tendency for K_i to rise and K_o to fall as a result, i.e. accumulation of potassium will occur. This problem is more connected with secretion than with ionic permeability, yet it is of interest to the student of the latter since Osterhout insists that the mechanism of penetration of potassium is not the migration of an ion in exchange for another from the inside, nor yet by the passage of two ions of opposite charge such as K^+ and Cl^- , but rather it is brought about by the passage of the undissociated potassium hydroxide, or alternatively by the combination of potassium with some acid membrane constituent

in the form of an undissociated complex. This view is apparently not shared by many workers other than Osterhout and his collaborators. Furthermore, as Osterhout has recognised, the electrical potentials observed across the membranes of these large plant cells can best be accounted for on the basis of ionic diffusion rates. The arguments put forward by Osterhout have been reviewed exhaustively by this author (1933), in a paper to which the

TABLE XXXV. CHEMICAL ANALYSES OF SAP OF VARIOUS CELLS, AND OF THE EXTERNAL MEDIUM NORMALLY SURROUNDING THE CELLS (OSTERHOUT, 1936)

	Sea water	Sap of <i>Valonia</i> <i>macrophylla</i> (Bermuda)	Sap of <i>Valonia</i> <i>centricosa</i> (Florida)	Sap of <i>Halimystis</i> <i>Osterhoutii</i> (Bermuda)
	M	M	M	M
Cl	0.580	0.507	0.608	0.603
Na	0.498	0.09	0.0348	0.557
K	0.012	0.5	0.576	0.0064
Ca	0.012	0.0017	Trace	0.0005
Mg	0.057	Trace?	Trace	0.0107
SO ₄	0.036	Trace?	Trace	Trace
H ₂ PO ₄
NO ₃

	Sap of <i>Nitella</i> <i>clavata</i> (California)	Pond water bathing <i>Nitella</i> <i>clavata</i>	Sap of <i>Chara</i> <i>ceratophylla</i> Finland	Brackish water bathing <i>Chara</i> <i>ceratophylla</i>
	M × 10 ³	M × 10 ³	M × 10 ³	M × 10 ³
Cl	90.8	0.903	225.0	73.0
Na	10.0	0.217	142.0	60.0
K	54.3	0.051	88.0	1.4
Ca	10.2	0.775	5.3	1.8
Mg	17.7	1.69	15.5	6.5
SO ₄	8.33	0.323	3.9	2.8
H ₂ PO ₄	3.61	0.0002	4.1	Trace
NO ₃	0	0.55	0.4	0.005

interested reader may be referred. The experimental evidence adduced in favour of the hypothesis is based mainly on the calculation of diffusion constants appropriate to the various possible mechanisms in terms of the ionic mobilities. Jacques & Osterhout (1934) have measured the rates of accumulation at two different pH and have argued that on the basis of ionic exchanges (i.e. an exchange of K⁺ with H⁺) the ratio of the rates should be 1.09, whilst by the penetration of undissociated potassium hydroxide the ratio should be 5.5; the observed rate was found to be 3.5, a fact which they interpret as a partial

confirmation of their theory, although the observed ratio lies almost midway between the two calculated values. A more serious objection to the simple treatment of accumulation in plant cells is the exception afforded by *Nitella*, with which cell it has been found that the rate of penetration of potassium is independent of pH between pH 6 and 8 Jacques & Osterhout, 1935, and further it has been found that potassium will enter the cell even when the product $K_1OH_1 > K_2OH_2$ see also Jacques, 1936.

An extremely careful and accurate study of the penetration of arsenic salts into *Valonia* is reported by M. M. Brooks (1925), who used chemical technique throughout; her results are in disagreement with Osterhout's views on salt penetration in that the rate of penetration is more nearly proportional to the concentration of ionised arsenic than the concentration of undissociated salt. A minimum rate of penetration was found at neutral reaction. This author points out that it is necessary to observe the plants for 1 month after experimentation to ensure that no damage has been done by the procedures used, and not merely overnight as Osterhout had done.

Qualitative evidence regarding the penetration of lithium, caesium and strontium has also been brought forward by this author (1922); the rates of penetration are apparently dependent on the proper balancing of the salt solution. So far as the relative rates of penetration of some ions are concerned, Damon (1932) has calculated relative mobilities in the protoplasm for K^+ , Cl^- and Na^+ , on the basis of purely electrical measurements, to be 20:1:0.2. Ullrich (1934) shows that when *Valonia* cells are placed in sea water, to which nitrate has been added, the latter penetrates the cells and in 5 days attains equal concentrations inside and outside; similar results are found with bromide and salicylate, the former ion migrating more and the latter less rapidly. Jacques (1937) has shown that iodine penetrates into *Valonia*, the kinetics corresponding to the migration as sodium iodide, as opposed to any exchange of ions.

Cooper *et al.* (1929), using a flotation method of studying ionic permeability, claim that the rates of penetration of potassium and sodium into *Valonia* are 3×10^{-8} moles per hour per sq. cm. of surface and 0.51×10^{-8} ; it is claimed that lithium, rubidium, bromine and iodine penetrate whilst thiocyanate, salicylate and nitrite do not. It is to be noted that the rates of penetration of

sodium and potassium are not defined in terms of concentration gradients or in terms of a driving force, whatever this may be, so that it is impossible to deduce mobilities from these figures.

Hoagland & Davis (1923) show that acidity of the medium surrounding *Nitella* increases the rate of penetration of nitrate, and these authors are of the opinion that exchanges of anions occur: they find that sulphate is absorbed more rapidly than chloride. Perhaps the most satisfactory, and also the most disturbing (so far as the results go) study of accumulation of ions in plant cells is that of Brooks; this author (1932), by means of direct chemical analyses, has shown that rubidium, when added to the solution bathing *Valonia*, penetrates into the sap. The rate of disappearance from the medium during the first 24 hours was 67×10^{-8} gm. moles per sq. cm. per hour, comparing with the values of 3×10^{-8} for potassium and 0.55×10^{-8} for sodium (Cooper *et al.* 1929). The rate of appearance of rubidium in the sap, however, was virtually independent of the concentration of rubidium outside the cell when this was varied between 1.3 and 5.3 millimoles per litre. Nevertheless, the amount removed from the solution depended on the amount initially present. This apparent contradiction was resolved by Brooks in a later paper (1935), when he showed that the protoplasm could not be viewed simply as a membrane, but that it was the initial seat of accumulation. It was found that protoplasm takes up rubidium at the rate of 1×10^{-6} gm. moles per sq. cm. per hour, whilst under the same conditions it appears in the vacuole at about 9×10^{-7} gm. moles per sq. cm. per hour during the first 2 days; at the end of this period the ratio of rubidium concentration in protoplasm to rubidium concentration in the outside medium (the accumulation ratio) becomes 39; then rubidium passes more rapidly into the sap and also back into the external medium, so that the protoplasm accumulation ratio falls rapidly and the sap ratio may rise. Continuing his investigations, Brooks (1938), by the use of radioactive potassium, has shown that the accumulation of potassium follows essentially the same lines as that of rubidium, the protoplasm being the initial seat of accumulation. A more complete investigation has appeared recently (Brooks, 1939). The main bearing of these results on the problem of ionic permeability is a negative one, in that they show how inadvisable it is to formulate theories of the mechanism of ionic diffusion on the basis of studies

which are confined to the sap and the surrounding medium: i.e. studies which ignore the role of the protoplasm.

Gollander (1939) has studied the uptake of ions by *Chara corallipes* and *Tolypellopsis stelligera* (abbreviated *Tol.*). The absorption of Li, K, Rb and Sr is stimulated by light. The uptake of lithium is greatly reduced in absence of oxygen, but pH changes of the medium between pH 5 and 8.4 had no very marked action. Exchanges of ions across the protoplasm, i.e. between the vacuole and the medium, were of the order of 10^6 times slower than that across an equal thickness of water for e.g. Na and K, Rb and K. In the whole resistance of the protoplasmic layer is due to the cell membranes, the effective thickness of the layer restricting diffusion is reduced from 10^{-3} cm. (the thickness of the protoplasmic layer) to 10^{-6} – 10^{-7} cm., reducing the permeability to 10^{-9} – 10^{-10} of that of an equal thickness of water.

The accumulation of ions by plant roots presents problems of interest to the student of permeability. If, however, it is realised that the plant lives in a medium extremely poor in all ions, it will be clear that absorption must be associated in general with a metabolic activity. In fact, the correlation between accumulation of ions and carbon-dioxide production in land plants is more obvious than in any other accumulation system, *vide* e.g. Lundegårdh (1937). Prevôt & Steward (1936), and investigators have properly concerned themselves mainly with the problem of this relation between metabolism and ionic penetration. Until something more is known about the driving force which determines the migration of an ion in these systems, it is not very profitable to discuss this migration from a simple permeability point of view. The interested reader may be referred to reviews of Steward (1937), Gregory (1937) and Hoagland (1937, 1940).

Yeast. A satisfactory study of the permeability of the yeast cell based on chemical methods is that of Paine (1912); this author found no evidence of penetration of sodium chloride into yeast after 3 hours at room temperature; after 20 hours in the cold store a small amount of penetration had occurred. Similar findings were reported for ammonium sulphate and an isotonic (0.10 *M*) solution of Na_2HPO_4 and sodium arsenate. If the concentration of Na_2HPO_4 was raised to 0.3 *M* a well-marked penetration was observed; no experiments were carried out to see whether this effect was one of altered tonicity or of pH.

Aquatic Animals. The classical work in this field is that of Loeb and his collaborators on the minnow, *Fundulus*. Loeb & Wasteneys (1911) had shown that *Fundulus* is rapidly killed by a pure potassium-chloride solution in the concentration in which it is present in sea water; however, if sodium chloride is added to the potassium solution such that the ratio of sodium to potassium is 17 or more to 1, the animals live indefinitely. These authors explained the results of the addition of sodium chloride as being due to the prevention of the diffusion of potassium into the animal where it exerted its toxic action. Loeb & Cattell (1915) continued this work, using the embryo of *Fundulus*. If the latter is transferred from sea water to pure potassium-chloride solutions the heart beat stops; on transferring it now to distilled water or to saccharose solution no recovery of the heart beat is observed, whereas transference to a solution of an electrolyte such as NaCl causes recovery of the embryo from the toxic effects of the potassium, due, as these authors assume, to the diffusion out of the ion to its surrounding medium. The efficiency of the electrolyte in promoting recovery was found to vary with the anion in the following series:

citrate > SO_4 > tartrate > acetate > I > Br > Cl, NO_3

(Table XXXVI), citrate being the most effective. The effect of cations was not so clear-cut; in general it was concluded that cations retard recovery, NH_4 , Rb and Cs having a greater effect than Li^+ and Na^+ , and Mg^{++} , Sr^{++} and Ba^{++} having a greater effect than Ca^{++} . Recovery was also found to be facilitated by addition of acid to the medium, whereas alkali was without influence.

The effect of addition of sodium salts on the poisoning by potassium chloride, as opposed to the recovery, gave a similar series of anions, shown below (Table XXXVII), sodium sulphate being more effective in preventing poisoning than sodium tartrate, which is more efficient than acetate, etc. If the effect of the added sodium salt is to prevent the penetration of potassium, then it follows that the anions behave differently according as the penetration of potassium into or out of the embryo is being studied. A further interesting point discovered by these workers was that a preliminary treatment of the embryos in distilled water made them temporarily immune to the toxic action of potassium

chloride. This point was further investigated by Loeb (1916*a, b*), who concluded that the diffusion of potassium into, and out of, the embryo depended on a certain "salt effect"; if the embryo was

TABLE XXXVI. THE INFLUENCE OF THE ANION UPON THE NUMBER OF HEARTS BEATING FROM TREATMENT WITH KCl IN M/3; SODIUM SALTS. TWENTY EMBRYOS WERE USED IN EACH BATCH. LOEB & CATTELL

After	Seawater	NaCl	NaBr	NaI	NaNO ₃
2½ hr.	9	2	3	4	2
4½ hr.	2	4	6	8	5
5½ hr.	5	9	8	10	11
9½ hr.	1	14	18	17	16

After	NaCNS	Na acetate	Na ₂ tartrate	Na ₂ SO ₄	Na ₂ citrate
2½ hr.	9	2	6	5	19
4½ hr.	4	6	12	13	14
5½ hr.	7	12	14	19	11
9½ hr.	"	19	19	20	Dead

TABLE XXXVII. EFFECT OF ANIONS ON THE POISONING OF *Fundulus* EMBRYO HEARTS BY KCl. TWENTY EMBRYOS WERE PLACED IN EACH SOLUTION. LOEB & CATTELL, 1915:

Number of hearts beating in 6.6 ml. M/2 KCl in 50 ml. of					
After	H ₂ O	M/4 sea water	M/4 NaCl	M/4 NaBr	M/4 NaI
1 hr.	11	19	18	18	18
4½ hr.	2	12	14	11	16
22 hr.	1	11	11	8	3
48 hr.	1	7	9	4	1

Number of hearts beating in 6.6 ml. M/2 KCl in 50 ml. of				
After	M/4 Na acetate	NaCNS	M/4 Na ₂ tartrate	M/4 tartrate
1 hr.	20	16	20	20
4½ hr.	15	3	10	19
22 hr.	13	1	18	16
48 hr.	14	0	18	16

washed with distilled water, then potassium would only be able to penetrate after it had exerted its own salt effect, i.e. its penetration would be delayed.

Loeb (1916*a, c*) showed too that the inhibition of the poisoning action of pure potassium-chloride solutions by a sodium salt was dependent on a definite proportion of sodium to potassium; if

this ratio was decreased below a certain limit it was found that the sodium salt actually accelerated the poisoning. Loeb considered that this fact accorded with the general notion of a preliminary "salt-effect" on the membrane by means of which diffusion of potassium is facilitated.

In a further paper (Loeb, 1916*b*), the effect of the cations in facilitating the recovery of poisoned embryos was reinvestigated with more definite results. It was concluded from this work that Li^+ , Na^+ , Mg^{++} and Ca^{++} all favour recovery, whereas there was practically no recovery if the embryos were transferred from their potassium-chloride solution to rubidium and caesium solutions; barium and strontium were also found to be very much less effective than lithium and sodium.

Loeb & Cattell interpret the results on the behaviour of *Fundulus* embryos on the assumption that the diffusion of potassium presupposes its combination with a colloidal anion of the membrane; it is further assumed that anions will inhibit this combination, especially divalent and trivalent ones.

However, it must be pointed out that no chemical determinations of potassium were made in any of these experiments, the sole criterion of penetration of this ion being the poisoning or recovery of the embryonic heart. Thus, the observation that rubidium and caesium salts do not facilitate recovery whereas lithium and sodium salts do, which Loeb interpreted as being due to the fact that the former salts inhibit the escape of potassium, might just as easily be explained by assuming that rubidium and caesium are themselves toxic to the heart. The complete explanation of Loeb's striking results must obviously await further investigation of a more exact nature based on the chemical determination of potassium; thus it is of primary importance to determine whether the *Fundulus* embryo is specifically cation permeable and impermeable to anions. Definite evidence on this point is lacking, in fact the simple osmotic equilibria between the *Fundulus* egg and its environment are not at all clear. Loeb & Wasteneys (1915) have shown that the osmotic pressure in the egg is much lower than that in the sea water (depression of freezing point about 0.76°C . compared with about 1.8°C . for sea water). Since the egg may be transferred to distilled water without swelling, it is likely that the membrane is impermeable to water, and also an impermeability to salts as such is indicated

by the ability of the embryo to develop in distilled water. Loeb's work indicates a permeability to potassium, and this is made more probable by the work of Ikeda (1937 *a, b*) on a closely related egg, that of *Oryzias latipes*, who demonstrated by direct chemical methods that potassium passes into the eggs against a concentration gradient: the figures presented by Ikeda have been criticised by Krogh (1939) and re-calculated, but the essential fact of this accumulation remains.

If the cell is permeable to cations and impermeable to anions, it is clear that potassium would not be able to diffuse in appreciable quantities out of the embryo when the latter is placed in distilled water or non-electrolyte solutions, just as Loeb observed. Similarly, the addition of acid to the distilled water or non-electrolyte solution, by providing the necessary hydrogen ions for the exchange of potassium across the membrane, would facilitate recovery also, just as Loeb observes. The effect of preliminary repeated washing of the embryos with water, whereby the poisoning with KCl solution is retarded, could also be explained on the basis that potassium is gradually washed out of the cell by exchanges with hydrogen ions from the water so that subsequent placing of the cells in KCl solution does not cause nearly so toxic an effect as normally, simply because the amount of potassium which must diffuse into the cell to reach a toxic concentration is greater as a result of the preliminary leaking out of potassium. Thus some of the interesting results of Loeb could be explained on this simple basis without invoking combination of the potassium with colloidal molecules: the effect of polyvalent anions in accelerating the escape and retarding the entry of potassium must be at least in part an influence on the membrane, and it is interesting to note that polyvalent anions accelerate the escape of sodium from the inside of the cat erythrocyte whilst they inhibit the penetration of potassium from the outside to the inside (Chapter XII).

A more recent investigation of the poisoning of *Fundulus* embryos is that of Bridges & Sumwalt (1934), without, however, any improvement of the technique of Loeb; these authors find that the rate of poisoning is decreased by increasing the acidity of the medium from pH 7.2 to pH 3.8; this is in contrast with the experiments on recovery in which acidity *increased* the latter. A rather disturbing paper on the same subject by Heilbrunn &

Daugherty (1934) claims that the effects of acidity have nothing whatever to do with permeability, but are rather to be associated with the changes in viscosity which are produced within the embryo by the altered reaction. Whatever may be the truth of the matter, it is clear that chemical studies must be the deciding factor.

Sumwalt (1933), on the basis of measurements of the potential difference across the *Fundulus* chorion, by insertion of a calomel electrode into the egg, claims that at normal reactions the membrane is more permeable to positive ions than to negative. At pH 3.4 equal rates of diffusion of potassium and chloride ions are claimed; however, it is difficult to believe that the cell is not damaged by such a large pH change.

While we are discussing the permeability of marine eggs, it should be noted that the most studied example in regard to permeability to water and non-electrolytes, namely *Arbacia*, has been comparatively neglected so far as ionic permeability is concerned; this egg is apparently normally impermeable to salts, since the kinetics of swelling in diluted sea water indicate no significant loss of osmotic material. In non-electrolyte solutions, however, Moore (1932) has demonstrated a continuous leakage of salts from the *Arbacia* egg. Whether this escape is sufficient to prejudice the osmotic studies of Lucké and his co-workers cannot be discovered, since Moore produces no experimental figures on this point; the matter certainly warrants further investigation, since the work of Lucké on antagonism in regard to water permeability was carried out in virtually electrolyte-free solutions. Chemical evidence (Page, 1927) indicates that the potassium concentration is considerably higher in the egg than in the surrounding sea water. Jacobs & Stewart (1936), from studies of the penetration of ammonium salts into the *Arbacia* egg, incline to the view that the egg membrane is impermeable to anions, or at any rate very much less permeable than with the erythrocyte membrane; Shapiro & Davson (1941) suspended *Arbacia* eggs in sea water and found a definite leakage of potassium out of the cell; they also showed that increasing the potassium content of the sea water caused a slow entry of potassium into the eggs. The main difficulty in the way of studies of ionic permeability in marine eggs is the limited season during which they are available; chemical methods are laborious and involve the use of large quantities of eggs.

Krogh *et al.* 1938 have studied the permeability of the egg of the Teleosts *Pleuronectes fjesus* and *Crenilabrus exoletus* to salts and water. While in the oviduct these eggs are in osmotic equilibrium with the surrounding fluid and hence with the blood of the fish. On shedding into sea water it was found that both penetration of chloride into and loss of water from the eggs occurred. This is apparent from Table XXXVIII, in which the chloride content per 100 eggs and the chloride content per 100 mg. of eggs are shown in freshly shed eggs and in eggs which have been placed in 15, 25 and 34‰ sea water. If the exchanges had been confined to water shifts from the more dilute to the more concentrated solutions, i.e. from the egg to the sea water, then the chloride per 100 eggs should have been unchanged; since this was not the case, one concludes that chloride penetrated the eggs.

TABLE XXXVIII. KROGH, KROGH & WERNSTEDT, 1938

	100 eggs		100 mg.		Sea water %Cl per 100 mg.
	mg.	Cl- %	No.	Cl- %	
Fresh from fish	73.0	6.4	137	8.8	
In 15‰ sea water	62.1	12.5	161	20.2	25.0
After 6 hr.	69.2	19.0	145	28.2	
In 25‰ sea water	72.0	23.8	139	33.4	42.0
After 6 hr.	69.5	25.8	144	37.0	
In 34‰ sea water	60.3	17.9	166	29.7	55.5
After 6 hr.	65.2	26.7	152	46.7	

The eggs of fresh-water organisms such as the trout or salmon have been studied fairly extensively owing to the special nature of the problems connected with the maintenance of the integrity of an egg cell when it is laid in fresh water. The interested reader must be referred to the original publications, among others those of Gray (1920, 1932), Runnstrom (1920), Svetlow (1929), Manery & Irving (1935) and Krogh & Ussing (1937), for the many interesting details associated with these eggs. It is sufficient for our purposes to note that the egg in the female is in osmotic equilibrium with the latter's blood. On being shed into fresh water the egg swells initially, due to osmosis of water—no salts are lost, however; during the swelling process, which occupies less than 1 hour, the chorionic membrane becomes tough and a new membrane, called by Gray the vitelline membrane, forms at the surface of the egg proper; as a result of the swelling a space between this vitelline membrane and the chorion, filled with

fluid, appears (the perivitelline space, and this fluid takes up the composition of the surrounding medium. After 1 hour no more swelling occurs and it is generally agreed that this is due to an absolute impermeability of the vitelline membrane to water.

The worm *Gunda ulvae* presents some interesting features from the point of view of the modification of the membrane's permeability to water and salts by changes, occurring naturally, in its ionic environment. This worm lives in estuaries and as a result of tidal action the medium surrounding it varies from sea water

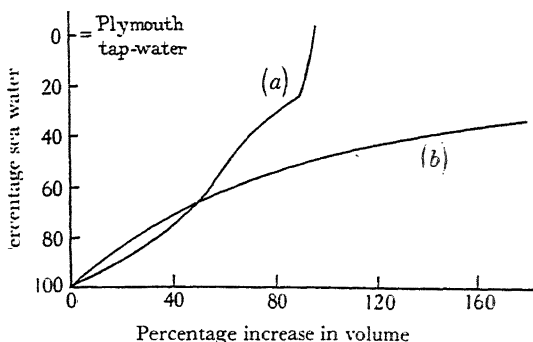


FIG. 44. Changes in the volume of *Gunda ulvae* after 75 min. in various solutions: (a), experimental curve, (b), theoretical curve for an organism bounded by an ideal semi-permeable membrane.

to completely fresh water. Pantin (1931) and Weil & Pantin (1931) have made an interesting study in an effort to show in what way the worm is able to adapt itself to these large changes; in Fig. 44 is shown the change in the volume of *Gunda* when it is placed in diluted sea water; it is evident that the worm swells as a result of osmotic intake of water, yet the swelling is not large enough if one assumes that the membrane separating *Gunda* from its surroundings is impermeable to salts; hence an escape of the latter is presumed, which was confirmed by conductivity measurements. In tap water it was found that the worm dies; yet addition of a little calcium to this water enables it to survive. It seems from Pantin's work that the presence of calcium not only decreases the permeability of *Gunda* to water, but also to salts. Nevertheless, it is found that, as a result of diffusion of water into the worm and of salts out, the concentration of salts in the worm is reduced to

about $6 \cdot 10^{-4}$ of the original. When this dilution is reached no further volume changes occur, indicating an active excretion of the absorbed water as it penetrates (Beadle, 1931).

Before leaving the subject of the permeability of aquatic animals to salts it would be of interest to discuss two examples of the adaptation of an adult organism, with a thick integument, to its ionic environment: it might be expected that the skin of a fish, for example the gold-fish, would be impermeable to salts, all ionic exchanges with the fish and its environment being expected to occur across the gill membrane. However, this is by no means the case, and it is found with the gold-fish that it can be readily depleted of its salts by the simple expedient of continual washing with running water. Hence we must conclude that the adult fish has a mechanism of absorbing salts from its environment, and in the case of the fresh-water species, this absorption must be against a concentration gradient. Krogh (1938) has shown that with the gold-fish *Carassius auratus*, chloride can be absorbed by the gills from sodium, potassium, ammonium and calcium chlorides, and from mixtures of these salts with nitrates and iodides. Bromide is also actively absorbed, whereas, although iodide penetrates slowly by diffusion, it is not actively absorbed; nitrate and thiocyanate are neither actively absorbed nor do they penetrate by simple diffusion. There is apparently, also, an independent mechanism for absorbing cations in respect to sodium; however, potassium is not actively absorbed. The skin of the amphibian, the frog, has been the object of numerous investigations both in respect to ionic and water permeability; in particular, Wertheimer

1923-1925 has studied the so-called irreciprocal permeability to dissolved substances. By this is meant the more rapid migration of a given substance in one direction than in the opposite. However, as Krogh (1939) very aptly points out, "much of the work has given conflicting results and the interpretation is exceedingly difficult, because the power of the skin to absorb ions was unknown and often brought about experimental conditions which could not be reproduced". If we confine ourselves to the strictly controlled experiments of Krogh (1937) we note that the frog skin behaves in an essentially similar manner to that of the gold-fish in that salts may be washed out of the animal by simply spraying the skin with distilled water; the skin of this animal, like the gills of the gold-fish, is capable of the active absorption of ions

in approximately the same manner as the latter, the permeability to ions, however, being on the average higher; once again it was noticed that potassium was not absorbed and also no penetration by diffusion was observed. We have already seen in the chapter on ionic permeability in the erythrocyte that the classical notions regarding potassium and sodium permeability (the latter being considered to be less than the former owing to the greater ionic diameter) must be revised, and it is interesting that Krogh's work on gill membranes and frog skin would also demand a reconsideration of these views.

Muscle and Nerve. We have already discussed in some detail the qualitative aspects of the permeability of the muscle fibres to certain ions with special reference to the problem of the normal ionic and osmotic equilibria pertaining between the muscle cells and the interstitial fluid (p. 35). The general picture presented was that of a cell impermeable to sodium and anions and permeable to potassium. These ionic relationships are of exceptional importance, since it seems that they are the basis of the resting potential observed by placing electrodes on a damaged part of the tissue and on an intact part, the view being that as a result of the tendency of potassium to diffuse out of the muscle cell the latter is negative in respect to its surrounding medium. If this is the correct picture, it is clear that a study of this potential under a variety of conditions, or alternatively a study of the potential obtained by placing one electrode on a normal piece of muscle and the other on a part which has been treated with some special solution to alter the normal permeability relationships at that point (the so-called salt effect), will provide information respecting the permeability of the muscle fibres to various ions. The interested reader is referred to the monograph by Höber (1922) for an elaborate description of the results of this sort of measurement.

A general impermeability of the muscle membrane to certain salts was first indicated by the work of Overton (1904), who found that there was no increase in weight of isolated muscles when soaked in 0.7% sodium chloride; by reduction of the concentration to 0.6% the muscle swelled, whilst increase to 0.9% caused a shrinking. Isotonic solutions of potassium salts could be divided into two classes: one, consisting of the chloride, bromide, nitrate and iodide, caused an irreversible swelling, whereas the

consisting of the sulphate, phosphate, tartrate, ethylsuccinate and acetate, allowed the muscle to maintain its normal weight. Rubidium salts behaved in the same manner. Isotonic calcium chloride caused an irreversible shrinking, but a mixture of sodium and calcium chlorides enabled the muscle to maintain a constant weight.

The interpretation of Overton's results was, and still is, difficult: in general, however, they go to prove that the normal permeability relationships of the muscle cell are dependent on a suitable ionic milieu, just as with plant cells; the swelling in pure solutions of some of the potassium salts would indicate a breakdown of the normal impermeability to salts, either by making certain anions permeable or allowing the migration of sodium and anions, so that the colloid osmotic pressure of the cell contents came into play, bringing about the penetration of water into the cells.

Urano 1908 and Fahr 1908, by direct chemical methods were able to show that only 6% of the total muscle potassium was washed out with sugar solutions, whereas 90% of the sodium was removed. These authors were of the opinion that the sodium was mainly present in the tissue fluid and was therefore washed out with ease: they assumed that the muscle fibres were impermeable to potassium.

Stanton 1923 perfused frog muscles with potassium-free Ringer solution and found that potassium leaked out of the perfused muscle; thus about 3% of the total potassium was lost in 30 min., about 8% in 4 hours and about 17% in 16 hours. This author found no significant change in the amount which diffused out in a given time on changing the reaction of the perfusion fluid from pH 6 to pH 8, yet an increased rate at the more acid reaction might be expected since the increased concentration of hydrogen ions would facilitate ionic exchanges. However, Fenn & Cobb (1934, 1935) have shown that the equilibrium concentration of potassium in isolated muscles varies with the pH of the medium; if the muscle is in Ringer solution, increasing the CO₂ tension increases the amount of potassium in the outside fluid, owing to the fact that the Ringer solution has less buffering power than muscle and its pH shifts more to the acid side than does the muscle pH; if the muscle is placed in serum the reverse process occurs, since in this case the buffering power of the serum is the greater.

Mond & Amson (1928), by a perfusion technique, have shown that the muscle fibres are impermeable to calcium, lithium and sodium but permeable to potassium and caesium. These authors showed the necessity for maintaining a definite concentration of potassium in the perfusion fluid, above which potassium entered the muscle cells and below which it leaked out; the concentration was found to be about 13 mg. %, a point which has been amply confirmed by the determinations of Fenn & Cobb (1934). The permeability of muscle cells to rubidium and caesium besides potassium is made probable by the work of Mitchell *et al.* (1921), in which rats were fed these elements. It was found on post-mortem examination that about 50% of the potassium in the muscle had been replaced by either rubidium or caesium. By perfusion of frog's muscle, however, they were only able to demonstrate penetration of rubidium and caesium if the muscles were stimulated. All the work quoted so far indicates, then, that the muscle is permeable to potassium; quite recently, however, Hahn *et al.* (1939) have published some results of experiments with radio-active potassium which would apparently indicate that the large proportion of potassium in the muscle cells is unable to diffuse out. These authors injected radio-active potassium K^{42} , into rabbits and frogs and found that the amount of this element in the muscle was about twenty times less than if a free exchange between the normal potassium and its radio-active isotope had occurred; rather similar results were reported independently by Joseph *et al.* (1939) on rat muscle.

If the muscle cell is assumed to be permeable to potassium, then we must assume that it is impermeable to sodium, unless there is some active secretory mechanism involved, since the normal muscle cell contains almost exclusively potassium whilst the interstitial fluid in contact with the cell contains almost exclusively sodium. However, Heppel (1939) has maintained rats on a reduced potassium diet and has found that by this means the muscles may be depleted of their potassium to the extent of about 50%; this depletion is made good by sodium, a fact which indicates that the muscle cell can, under certain conditions at least, allow the penetration of this ion. By injection of radio-active sodium into these rats it was found that the isotope is equally distributed between plasma and muscle within 60 min.; Manery & Bale (1939), using normal rats, have also demonstrated a rapid

interchange of radioactive sodium with the small quantities of sodium normally present in muscle cells.

The normal impermeability of the muscle cell to anions, which must be invoked to explain the absence of appreciable quantities of chloride in the muscle cell and also the failure of sodium to diffuse into the cell and of potassium to diffuse out,* is supported by the observation that thiocyanate is not taken up by a perfused muscle when this ion is added to the perfusion fluid Mond & Anson, 1928, by the observations of Conway & Kane 1934, who found that phosphate and sulphate were not distributed throughout all the water in the muscle, and the experiments of M.G. Egeleton 1933, which confirmed the behaviour of muscle towards phosphate. The failure of iodide to partition itself with more than 30% of the muscle water observed by Ghaffar 1935, would indicate an impermeability to this ion also. It seems to be generally agreed that lactate diffuses throughout all the water in muscle, but this is probably due to the penetration of the membrane as the undissociated acid.

The effects of the activity of the muscle on its ionic permeability have been extensively studied. Mitchell & Wilson (1921) found no increase in the rate of loss of potassium from muscle perfused with potassium-free Ringer solution when it was stimulated; Ernst & Scheffer 1928, showed that stimulation of a perfused muscle caused a 30-50% loss of potassium, and Ernst & Czucz 1929, showed that this loss of potassium is accompanied by a gain in sodium and chloride and a loss of phosphate; they further showed that no such changes could be observed if the muscle were stimulated indirectly, an observation confirmed by Mond & Netter 1930. Ernst & Fricker (1934) found that indirect stimulation would cause a loss of potassium provided that the potassium in the perfusion fluid was raised to four times its normal value. Fenn & Cobb (1936) were also able to demonstrate in intact muscles that losses of potassium could only be obtained on direct stimulation. However, Fenn (1937) showed that if the indirect stimulation was carried out at such a frequency as always to elicit a contraction of the muscle with each stimulus, then in 1.5 hours about 6% of the muscle potassium would be lost; it seems that failure of earlier workers to obtain the loss of potassium with indirect stimulation was due to fatigue setting in at the neuro-

* See, however, p. 38, note added in proof.

muscular junction, which soon prevents contraction of the muscle.

The changes in the ionic content of muscles, stimulated for some time, have been most accurately followed by Fenn *et al.* 1938, and the water, potassium, sodium and chloride changes are shown in Table XXXIX taken from these authors' paper. The table shows that potassium is lost, whilst water, sodium and chloride are gained. In general, the greater the loss of potassium the greater the gain of sodium; nevertheless, the gain of the latter ion was always greater than the loss of potassium. The authors conclude that sodium penetrates in exchange for potassium; the increase in chloride is accounted for on the basis of the increase in the intercellular space and is equivalent to the excess of sodium over that exchanged with potassium (see also Malorney & Netter, 1936). Fenn *et al.* conclude that no significant change in calcium and magnesium occurs as a result of stimulation but that a slight loss of total phosphate occurs. All these changes are reversed after a recovery period of a few hours.

TABLE XXXIX. ELECTROLYTE CHANGES DURING STIMULATION IN CATS (W. O. FENN *et al.* 1938).

Exp. No.	Water		Potassium		Sodium		Chloride	
	Rest c.c.	Stim. c.c.	Rest mM.	Stim. mM.	Rest mM.	Stim. mM.	Rest mM.	Stim. mM.
1	291	384	37.8	34.3	10.8	18.5	6.0	7.5
2	291	345	41.5	34.7	8.1	16.2	5.2	9.3
3	297	387	41.9	38.6	8.6	17.3	5.7	10.9
4	297	382	44.0	42.2	7.1	14.7	4.7	11.0
5	288	338	41.5	34.6	9.2	14.1	5.3	8.1
6	318	401	47.6	40.8	11.2	20.4	6.5	9.6
7	298	372	44.6	40.6	8.4	15.1	5.5	9.0
8	331	408	47.9	38.6	8.3	24.8	6.4	12.7
9	279	352	41.1	39.6	7.2	14.0	4.2	9.6
Av.	299	374	43.1	38.2	8.8	17.2	5.5	9.7
Av. change	+75		-4.9		+8.4		+4.2	
Av. in rats	+49		-6.1		+8.3		+2.8	

Nerve. The permeability of the nerve-cell membrane has not been studied to any great extent, presumably owing to the experimental difficulties involved. The comparative concentrations of potassium in crab serum and crab nerve have been reported by Cowan (1934), indicating that the potassium concentration of the nerve is about thirteen times greater than that in the serum;

that this high concentration of potassium might be the basis of the injury potential of nerve was suggested by Macdonald 1902, who showed that the potential could be modified by variation of the potassium content of the fluid surrounding the cell. A more detailed study of this nature was carried out by Cowan 1934, and this author made the exceedingly interesting observation that stimulation of the nerve caused leakage of potassium out of the nerve, shown by direct chemical determinations; some typical results are shown in Table XL. Netter 1927, by measurements of changes in the resting potential of frog nerve produced by immersion in different salts, concluded that the membrane is anion-impermeable and that the mobilities of the cations varied in the order: $\text{Ca} < \text{N} < \text{C}_2\text{H}_5 < \text{Li} < \text{Na} < \text{Cs} < \text{NH}_4 < \text{Rb} < \text{K}$, i.e. not necessarily in the order of their ionic radii; however, the interpretation of this sort of work is beset with many difficulties.

TABLE XL. THE LEAKAGE OF POTASSIUM SALTS FROM RESTING AND STIMULATED NERVES (COWAN, 1934)

A refers to the loss during the first 5 min. of stimulation, *B* during the second 5 min., and *C* during the first 5 min. following stimulation

Exp.	K leakage in mg. per min. per gm. of nerve		Frequency of of stimulation
	Resting nerve	Stimulated nerve	
2	0.0075	<i>A</i> 0.133	40 per sec.
		<i>B</i> 0.142	
		<i>C</i> 0.048	
4	0.006	<i>A</i> 0.131	40 per sec.
		<i>B</i> 0.145	
		<i>C</i> 0.064	
8	0.009	<i>A</i> 0.158	100 per sec.
		<i>B</i> 0.154	
		<i>C</i> 0.094	
10	0.0055	<i>A</i> 0.173	140 per sec.
		<i>B</i> 0.159	
		<i>C</i> 0.044	

When an impulse is carried down a nerve, electrical studies of the nerve at any point indicate the passage of a wave of electrical potential changes, known as the action potential; this action potential is presumed to manifest itself as a result of a change in the normal ionic permeability relationships between the nerve cell and its environment, whereby salts can move across the nerve cell membrane. Cole & Curtis (1939) have recorded the potential

and the membrane conductance (presumably a measure of the permeability of the membrane to salts) of the giant squid axon during the passage of a nervous impulse. It was found that the passage of the propagated electrical disturbance, as manifested by the action potential, is accompanied by a reversible change in the membrane conductance, indicating an increased permeability to salts.

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CHAPTER XIV

PERMEABILITY TO WEAK ELECTROLYTES AND DYES

By J. F. DANIELLI

PERMEABILITY TO DYES

THE advantages and disadvantages of the use of dyes for permeability studies have been very clearly analysed by Jacobs (1924). In the first place the penetration of dyes may be readily observed by optical methods. Secondly, dyes of a very wide variety of physical and chemical properties are available; they may have high or low oil-water partition coefficients, be acidic or basic, form crystalloidal or colloidal solutions. Nevertheless, dyes have been comparatively little studied of recent years, probably largely because most dye molecules are only distantly related to compounds of physiological importance, and it is now realised that even minor variations in molecular structure may profoundly affect the rate of penetration into cells, so that as most workers are interested in obtaining results of physiological importance, substances chosen for study tend to be the closer relatives of those of natural occurrence. But historically the study of dyes has been of the greatest importance in developing the basic theory of the subject, and it is in this light that we shall look upon this field.

In his pioneering studies of the nature of the cell membrane, Overton (1900) used a number of aniline dyes. He found that all those dyes which readily penetrate cells are absorbed by fats and stain lecithin and cholesterol. He used this observation to support the view that the cell membrane is a thin layer of fatty material. Ruhland (1908-1914) found a number of dyes, such as thionin and malachite green, which do not stain cholesterol but nevertheless readily penetrate cells: and that other dyes, such as victoria blue and rose bengal, cannot enter cells, but still stain cholesterol. These results led Ruhland to reject Overton's hypothesis, and to bring forward the contrary theory that the cell membrane is a molecular sieve or ultra-filter, discriminating between molecules in virtue of their diameter, and composed possibly of protein material.

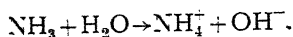
This explanation was not satisfactory to Höber (1922), who remarked that many dyes which are unable to penetrate plant cells enter animal cells with some ease, particularly in the case of colloidal fat-soluble dyes. Consequently the failure of a dye to enter a plant cell may be due to inability to penetrate the cellulose wall surrounding the plant cells. Furthermore, Herz (1922), studying *Opalina*, found that dyes such as thionin are not affected by the presence of anaesthetics, whereas penetration of the fat-soluble dyes is inhibited. Consequently, Höber suggested that the fat-insoluble and fat-soluble dyes enter by two different routes. With these facts and suggestions in mind, Ruhland's objections to Overton's hypothesis appear to be much less serious. Nierenstein's (1920) observations on *Paramecium* are also of interest. He found that there was no general correlation of ability of acid and basic dyes to penetrate *Paramecium*, with their partition coefficients in either acidic, basic or neutral oils, but a close correlation exists with the partition coefficients in an oil which contains both an organic acid and an organic base.

Of the more recent work, that of Irwin on the permeability of *Nitella* to cresyl blue is of outstanding importance. Cresyl blue is a basic dye, and penetrates rapidly when present as the free base, but very slowly when present as a salt. In fact it is possible that the salt cannot penetrate at all. At equilibrium the concentration of free base is the same both inside and outside the cell, but the amount of dye present as salt is greater on the side of greater acidity. Since the cell sap is acid (about pH 5), dye accumulates in the cell sap if the external medium is more alkaline than pH 5. Thus cresyl blue resembles the weak acids to be discussed later. Irwin (1925) has found a high Q_{10} for the penetration of cresyl blue into *Nitella*, and suggested that the dye must take part in a chemical reaction in passing through the cell membrane. This, however, may be a wrong conclusion (see p. 312). Commoner (1938) found a high Q_{10} for the entry of neutral red into *Chaetopterus* eggs. Whitaker (1936) found that changes in the permeability of sea-urchin eggs to dyes occur as a result of fertilisation. Ludford (1933) gives a review of many aspects of vital staining. Other aspects of this problem are covered by Scarth (1926), Cappell (1929), Salkind (1929) and Hall (1930).

PERMEABILITY TO WEAK ACIDS AND
WEAK BASES

The salts of strong acids and strong bases at physiological pH exist in aqueous solution entirely as charged ions. But the salts of weak acids and weak bases exist as a mixture of ions and of unionised free acid or base. A weak acid or base may thus penetrate a membrane either as an ion or as an undissociated molecule. Many physiologically important substances are weak electrolytes, e.g. H_2CO_3 , NH_4OH , lactic acid, adrenaline.

E. N. Harvey (1911, 1914) made a comparison of the ability of weak and strong acids, and weak and strong bases, to penetrate the same cell, using indicators. For example, a marine egg stained with neutral red is coloured red when in normal sea water. If $NaOH$ is added no change in colour appears until cytolysis occurs, so that as long as the cell membrane remains intact there is practically no penetration of alkali. If, however, a very small amount of ammonia is added to sea water, the cells rapidly become yellow, showing that alkali has reached the interior of the cell. Since the free OH^- ion present in $NaOH$ was unable to penetrate the cell, it follows that the alkalinity with ammonia must be due to penetration of NH_3 , which in the interior of the cell combines with water to produce hydroxyl ions:



By similar means Harvey showed that weak acids, such as butyric, acetic and carbonic acids, also penetrate cells as the free acid, and that both weak acids and weak bases may leave cells as readily as they enter.

Osterhout (1925) has studied the penetration of the weak acid H_2S into *Valonia*; the method being to expose the cells to sea water containing a constant amount of total sulphide, i.e. $H_2S + NaSH$, and measure the amount of sulphide present in the sap at equilibrium at various pH . The results are shown on Fig. 45. It will be seen that the total concentration of sulphide in the interior of the cell is proportional to the concentration of H_2S in the sea water, not to the total sulphide. The sap is at about pH 5, at which pH the sulphide is almost entirely H_2S , so that under Osterhout's experimental conditions we should find (concentration of sulphide in sap) = (concentration of free H_2S in sea water),

provided the cell membrane is permeable to H_2S only. Osterhout, therefore, concluded that, practically, only H_2S penetrates. Since the external total sulphide is constant, the external free H_2S decreases as pH increases, and consequently the H_2S in the sap decreases proportionately. But, if the *total* sulphide is varied in such a way as to keep the external H_2S concentration constant as the pH of the sea water is varied, we should find no change in the internal H_2S concentration as the pH is varied. This also was found to be the case by Osterhout. Essentially similar results were found by Osterhout & Dorcas (1925, for CO_2 , and by Irwin (1926) for the weak base cresyl blue; in both cases it is only the

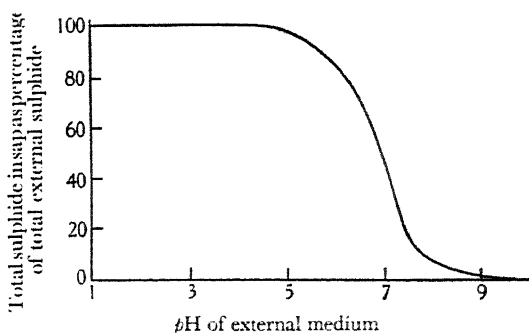


FIG. 45. Curve showing the relationship between the pH of a medium containing sulphide and the total intracellular sulphide in *Valonia*. (Osterhout, 1925).

unionised molecules which appear to penetrate. These studies are one of the few cases in which information about permeability may be obtained from the study of *equilibrium* conditions.

Further information has been obtained from observations on the kinetics of penetration of these substances. Jacques (1936) has studied the rate at which H_2S penetrates *Valonia*, finding that the number of molecules penetrating obeys, to a first approximation, the equation $x = a(1 - e^{-kt})$, where a is the external concentration of H_2S , x is the total sulphide (practically speaking all H_2S in the sap, t is time and k is a constant. This is the form of equation that should be obeyed if penetration is a simple process, not complicated by compound formation between H_2S and membrane molecules, and k should be independent of the absolute value of a . Fig. 46 shows that k is constant within experimental error.

In the case of bases, however, a quite different condition prevails. Since, as we have pointed out above, the equilibrium is between the free base in the sap and the free base in sea water, the rate of penetration of e.g. NH_3 should be proportional to the

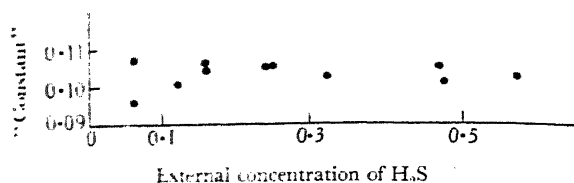


FIG. 46. Plot of the average velocity constant (from the first to the fifth minutes) for each equilibrium concentration of H_2S , the latter being regarded as equivalent to the concentration of molecular H_2S in the external solution. Jacques, 1937.

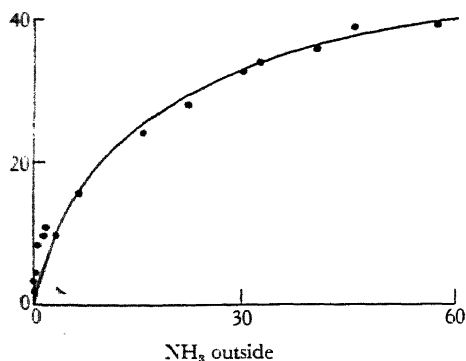


FIG. 47. Rate of entrance of NH_3 into *Valonia*, plotted against external concentration (Osterhout, 1936).

difference of NH_3 concentration of the sea water and the sap. Actually, as is shown by Fig. 47, the rate of penetration is not proportional to $[\text{NH}_3]_o - [\text{NH}_3]_i$, but falls off as the concentration difference increases (Osterhout, 1936). Osterhout suggested that actually the NH_3 combines with an acid constituent of the membrane, $\text{NH}_3 + \text{HX} = \text{NH}_4\text{X}$, and that it is this compound which diffuses across the membrane. In this case the rate of penetration will be proportional to the gradient, not of NH_3 , but

of NH_4X . If $[\text{NH}_3]_i = 0$, as is the case at the beginning of an experiment, we have

$$\Delta[\text{NH}_4\text{X}] = [\text{NH}_3]_o [\text{HX}] - \Delta[\text{NH}_4\text{X}] \frac{1}{k},$$

where k is a constant and $\Delta[\text{NH}_4\text{X}]$ is the concentration of NH_4X at the outer surface of the membrane. The full line of Fig. 47 was calculated from this equation, using $k = 0.00153$ and $[\text{HX}] = 0.005 \text{ M}$. In other words, the variation of the rate of penetration as the concentration gradient of NH_3 is increased is consistent with the view that the diffusing unit is not NH_3 but NH_4X . Essentially similar conclusions have been reached by Jacques for the penetration of the stronger base guanidine into *Valonia*. Poijärvi (1928) has made a study of the rate of penetration of a number of organic bases into plant cells, finding that lipoid solubility favours rapid penetration, and that strong bases tend to penetrate more slowly than weak bases of otherwise similar structure.

Gray (1922) has drawn attention to the physiological importance of the rapid penetration of weak acids and weak bases, pointing out that frequently such compounds have a more immediate and more profound action on the behaviour of cells than an equal concentration of a strong acid or strong base, and that recovery from the action of a weakly ionising substance is usually more rapid than from a strongly ionising substance.

Bodansky (1931) has studied the haemolytic action of acids added to isotonic NaCl , finding that the rate of haemolysis increases with the length of the hydrocarbon chain with the series of aliphatic acids $\text{C}_n\text{H}_{2n+1}\text{CO}_2\text{H}$. The haemolysis is probably due to an action of the acids on the cell membrane. It cannot be proportional to the rate of penetration of the acids, for the equilibrium conditions of red cells are not such as will lead to haemolysis in isotonic NaCl solutions containing a second penetrating component (see Chapter III). Nevertheless, this work is frequently quoted as evidence that permeability to fatty acids increases with chain length.

A special case of some importance is that of penetration of the ammonium salts of weak acids. In this case both anion and cation can penetrate in an unionised form. Jacobs (1922, 1924) made the first analysis of the behaviour of these salts, coming to the conclusion that NH_3 formed by hydrolysis is the

ammonia penetrates cell membranes, and finding that, in the case of red cells, haemolysis will occur with the NH_4 salts even of strong acids. This is because red cells are normally permeable to anions, so that in the case of, say, NH_4Cl , ammonia will penetrate as NH_3 , and chloride as the ion, so that there is no impermeable substance present to prevent the red cells swelling and bursting. Orskov (1934) claimed that the red cell is more permeable to NH_4^+ ions than to NH_3 , but this was regarded as highly improbable by Jacobs & Parpart (1938).

If we investigate the rate of penetration of the ammonium salts of different acids into the same cell, any differences observed between the salts must be due partly to the differences in the acid radicles. Thus in this way Jacobs (1927) found the rate of penetration of ammonium salts into red cells is formate < acetate < propionate < butyrate < valerate. Stewart (1931) found the same series with *Arbacia* eggs, as did Bouillenne (1930) with cells of *Tradescantia* and *Allium*; but Ruhland *et al.* (1932) with the bacterium *Beggiatoa mirabilis* found the series reversed, i.e. that valerate penetrates much more slowly than formate. Höber (1936) confirmed Jacob's observation on red cells and also found that the ammonium salts of aromatic carboxylic acids, which can penetrate as unionised acids, penetrate much more rapidly than the salts of aromatic sulphonic acids, which can only penetrate as the anion. Maizels (1937), studying the *exchange* of anions across the red cell membrane, found at $p\text{H}$ 5.1 the series formate < acetate < butyrate < valerate, and concluded that at this $p\text{H}$ penetration as the neutral molecule is of importance even with the red cell. At $p\text{H}$ 7 there was much less evidence of penetration as neutral molecules, and Maizels found little difference between formate and valerate. The theory of penetration of ammonium salts has been more quantitatively discussed by Jacobs (1940). Jacobs points out that even small changes of $p\text{H}$ are of the greatest importance when dealing with weak electrolytes, since the equilibrium between the various forms of the electrolyte is controlled by the $p\text{H}$. E.g. with a weak electrolyte HR we have

$$p\text{H} = pK' + \log \frac{[R^-]}{[HR]} \quad \dots\dots (29)$$

$$= pK + \log \frac{\alpha}{1 - \alpha} \quad \dots\dots (29.1)$$

Here K' and K are constants, and α is the fraction of the weak electrolyte which is ionised. To a first approximation pH may be regarded as the negative logarithm of the hydrogen-ion concentration. Then from (26.1) we obtain

$$\begin{aligned} [HR] &= (1-\alpha) C = \frac{C}{1+10^{pH-pK}} \\ \text{and} \quad [R^-] &= \alpha C = \frac{C}{1+10^{pK-pH}} \end{aligned} \quad \dots\dots 29.2 *$$

where C is the total concentration of weak electrolyte in all its forms. The net rate of entry of the unionised molecule into a cell is proportional to

$$\frac{C_o}{1+10^{pH_o-pK}} - \frac{C_i}{1+10^{pH_i-pK}} \quad \dots\dots 29.3$$

At equilibrium the two terms of (27) will be equal and the accumulation ratio C_i/C_o is

$$\frac{C_i}{C_o} = \frac{1+10^{pH_i-pK}}{1+10^{pH_o-pK}} \quad \dots\dots 29.4$$

where suffixes i and o refer to the inside and outside of the cell. In practice pH_i will frequently not remain constant, but will change according to whether the penetrating undissociated form is an acid, e.g. H_2CO_3 , or a base, e.g. NH_3 , as has been pointed out above (Harvey, 1911; Jacobs, 1922).

Jacobs criticises the view that the rate of haemolysis in isotonic NH_4 salt solutions is proportional to the permeability of the cells to anions. The mechanism which is assumed to operate in these cases is that the ammonium ion dissociates into $NH_3 + H^+$, penetrates as NH_3 , then combines with water to give $NH_4^+ + OH^-$; finally OH^- exchanges with Cl^- or other anion across the red cell membrane. But whilst the rate of exchange is proportional to the rate of penetration of the external anion, it is also proportional to the OH^- concentration inside the cell, which Jacobs points out is so low that it may very profoundly reduce the rate of anion exchange. Hence the rate of haemolysis is also a function of the pH inside the cell.

* Jacobs also points out that, following Brönsted, it is very convenient to use the same equations for weak bases, treating the ion (e.g. NH_4^+) as a weak acid which dissociates into $NH_3 + H^+$. In this case

$$\begin{aligned} [NH_4^+] &= (1-\alpha) C, \\ [NH_3] &= \alpha C. \end{aligned}$$

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CHAPTER XV

IMPEDANCE AND POTENTIAL MEASUREMENTS AND PERMEABILITY

By J. F. DANIELLI

IMPEDANCE MEASUREMENTS AND PERMEABILITY TO IONS

FROM alternating current impedance studies we can measure the electrical capacity and resistance of a membrane. We may assume that "the capacity is probably a property of the ion impermeable aspect of the membrane, while the conductance is due to the ion permeability" (Cole & Curtis, 1938). Consequently for us the main feature of interest is the resistance, which gives a measure of the permeability of a membrane to ions; there are, however, a number of interesting points about the capacity values.

The earliest measurements made on cells are those of Höber (1910-1913) on the red cell. Höber found that the interior of the cell had a high conductivity, but that the conductivity of the cell membrane is very low. The resistance of red cells as a whole, i.e. when the resistance was measured by direct current so as to include both the cell membrane and the cell contents, was approximately equal to that of 0.02% NaCl. But when fairly high-frequency alternating current is used to measure the resistance the effect of the membranes can be eliminated, and by this means Höber was able to show that the resistance of the interior of the cell was much lower, equal to that of 0.4% NaCl. Consequently, the high resistance measured with direct current must be due to the cell membrane. Höber's work on the red cell has been greatly extended by Fricke and his colleagues (1924, 1925, 1935) and McClendon (1926). The most interesting result so far obtained from the more accurate studies is a calculation from the capacity of the membrane of a value for the membrane thickness. Assuming a value of 3 for the dielectric constant of the membrane, it can be shown that the membrane thickness lies between 30 and 50 Å. (3-5 m μ). There has been a great deal of discussion as to whether 3 is the correct value to assume for the dielectric constant of the membrane. If the membrane is composed

of large fatty molecules such as triolein and cholesterol, 3 would be a reasonable assumption (Danielli, 1935). If, on the other hand, it is made of such molecules as guaiacol, the dielectric constant would be as large as 12 or more (Osterhout, 1937), and in this case the thickness would be of the order of 150 Å. Similar measurements have been made on many other cells, e.g. yeast cells and polymorphonuclear leucocytes (Fricke & Curtis, 1934*b*, 1935*a*), the eggs of *Arbacia*, *Asterias* and *Hippuris* summarised by Cole, 1937, *Valonia* and *Nitella* (Blinks, 1930, 1936; Cole & Curtis, 1937, 1938), and squid nerve axons (Curtis & Cole, 1938; Cole & Hodgkin, 1939). More recent results on *Arbacia* eggs, whole and after centrifuging into halves, and on *Cumingia* and *Chaetopterus* eggs, are given by Cole & Curtis (1938). All these studies are agreed in finding that cell membranes have a "static" capacity of about 1 microfarad per cm.², giving values for the thickness of the cell membrane in substantial agreement with those found for the red cell, i.e. between 30 and 150 Å., according to the value assumed for the dielectric constant.

Where the electrical resistance has been measured, it appears to lie between 1000 and 1,000,000 ohms per cm.² of membrane. This value applies only to normal cells in the resting state. *Nitella* membranes have an average resistance of 250,000 ohms per cm.², and for squid nerve the value is 10,000 ohms per cm.² We may compare these resistances with the specific resistance of various substances: *N*/10 KCl, 10² ohms; methyl alcohol, 10⁶ ohms; olive oil (dry), 10¹² ohms, and wet with salt solution, ca. 10⁷ ohms; paraffin, 10¹⁶ ohms; guaiacol (wet with conductivity water), 2.5×10^7 ohms. If we divide the resistance of the cell membrane by the specific resistance of the material of which it is composed, we should obtain a value for the thickness of the membrane. E.g. a membrane of resistance 10⁴ ohms per cm.², if composed of olive oil, would have a thickness of $10^4 \div 10^{12} = 10^{-8}$ cm. or 100 Å., a very possible value for the cell membrane; but if consisting of guaiacol, the thickness would be $10^4 \div 2.5 \times 10^7 = 4 \times 10^{-4}$ cm. or 4 μ , an improbably large thickness.††

This high resistance of 10⁴ ohms reflects the fact that the cell membrane has an extremely small permeability to ions, which diffuse across the cell membrane under an applied potential in about the same time as they would move from one side to the other of a layer of *N*/10 KCl 100 cm. in thickness. Alternatively,

comparing equal thicknesses of $N/10$ KCl and cell membrane, we find that the cell membrane is about 10^9 times less permeable to ions than the KCl solution.

However, these calculations which we have been making from resistance and capacity measurements rest upon a great many assumptions, and must be regarded with considerable scepticism until we have available studies of the resistance and capacity of thin artificial oil films of known structure and composition.

The high resistance (or low permeability to ions) of the surface of cells is of very great physiological importance, for it enables cells to maintain an interior ionic composition which may be quite different from that of the exterior medium. For example, most cells maintain an interior concentration of K^+ much in excess of that of the exterior medium; for muscle and nerve the ratio of interior to exterior concentration is about 20:1. For red cells there are variations from species to species, as shown by Table XLI. Where there is an excess of K^+ in the interior of a cell, it is usually balanced by an osmotically equivalent deficit of Na^+ or other cations. Owing to the low permeability of the cell membrane to cations, the red cell is able to maintain these concentration differences without the expenditure of a great deal of energy.*

TABLE XLI. SHOWING THE CONCENTRATIONS OF K^+ IN THE PLASMA AND RED CELLS OF VARIOUS SPECIES, IN PARTS PER THOUSAND BY WEIGHT

Species	Plasma	Red cells
Horse		4.1
Pig	0.27	5.0
Dog	0.25	0.27
Cat	0.26	0.26

Similarly, death of a cell is reflected by a dramatic increase in permeability to ions, as may be shown in many ways. For example, a living trout egg is impermeable to salts, and its contents are a clear pale yellow; after death salts leak out rapidly, and the yolk globulins are precipitated, giving the cell a cloudy white appear-

* Increasing the permeability to ions of a cell membrane may necessitate the performance of greater work by the cell to maintain its normal ionic composition. In view of this it is interesting to note that when the K^+ concentration of the Ringer bathing a muscle is raised, so is the rate of heat production (Solandt, 1936). Raising the K^+ concentration of Ringer is generally believed to increase permeability of excitable cells to ions.

ance. Again, chemical methods may be used to show that salts leak out rapidly only after death of the cell; see for example the work of Cowan (1934), who showed that K^+ only leaks out of asphyxiated nerve cells after asphyxiation has been prolonged sufficiently to cause irreversible damage to the nerve. But of all methods of showing this change, probably the most elegant is measurement of the electrical resistance of the cell, as was shown by Osterhout (1914, 1919), by experiments on *Laminaria* and frog skin. Osterhout measured the resistance of the tissues when the ionic composition of the bathing medium was changed from the normal. Table XLII shows a typical set of results with *Laminaria*: the resistance falls off continuously as the condition of the cells deteriorates, and finally reaches a value typical of dead cells in NaCl. In KCl solution the change of resistance of *Laminaria* was similar, but with isotonic $MgCl_2$ and $CaCl_2$ there was a rapid rise in resistance, followed by a fall to the value for dead cells. Osterhout states that he obtained similar results with frog skin, but Hogben & Gordon (1930), using a rather more accurate method, were unable to find confirmation of the details of these experiments when working with *Xenopus* skin, *Xenopus* ventral abdominal muscle, skin of *Rana fuscigula*, uterus of the viviparous Cape dogfish (*Acanthias*), and rabbit diaphragm. The conclusion that the resistance falls off as the cells die is, however, uncontested.

TABLE XLII. CHANGE IN OHMIC RESISTANCE OF *LAMINARIA* AFTER EXPOSURE TO ISOTONIC NaCl. RESISTANCE OF DEAD TISSUE = 320 OHMS

Time (min.)	Resistance ohms
0	980
20	745
40	590
60	495
150	345
300	320

Some interesting work on bacteria has also been published by Shearer (1919), Green & Larson (1922) and Zoond (1927). *B. coli* is stable in Ringer solution, and a suspension in Ringer has a resistance which does not vary with time (see Fig. 48). In a similar suspension in isotonic NaCl the resistance falls off with time, eventually reaching a value characteristic of the NaCl solution. $CaCl_2$, on the other hand, raises the resistance of the suspension. Shearer attributes this mainly to changes in the

resistance of the cell membrane. According to Green & Larson, and Zoond, this fall in resistance is due to leakage of salts from the cells and is no indication of a resistance change in the cell membrane. This explanation, however, cannot account for the *rise* in resistance which occurs after addition of CaCl_2 , nor for the fact that the fall in resistance at death occurs even with suspensions in isotonic saline; furthermore, the latter author's experiments are far from being exact repetitions of Shearer's experiments, since they have used more dilute suspensions and more dilute saline. In short, we are inclined to think that Shearer's results are to be

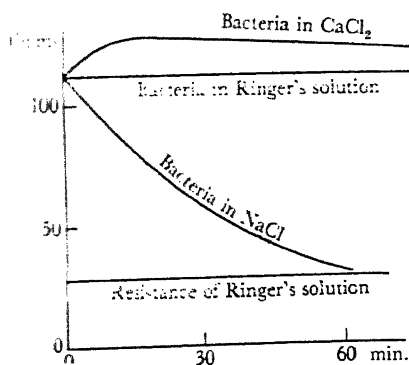


FIG. 48. The change with time in electrical resistance of suspensions of bacteria in solutions of various salts (Shearer).

explained largely in the manner he suggests, and that later workers have criticised his results after performing rather different experiments.

From these various impedance studies there is a certain amount of evidence that (a) CaCl_2 increases and NaCl decreases the resistance of the cell membrane, and (b) that in the absence of divalent cations the permeability of the cell membrane to ions is increased. With regard to (a), it may certainly be said that the initial action of Ca and Na on the observed resistance is consistent with this conclusion, but beyond this we cannot at present go owing to the complexity of the tissues used. For example, it is perfectly possible that the initial action of Na^+ and Ca^{++} on the tissues is really not on the cell membranes, but on the intercellular

matrix, which according to Gray (see Chapter VI of his *Experimental Cytology*), is often very sensitive to the character of the ionic environment; thus, according to Herbst, in Ca-free sea water the intercellular matrix of Echinoderm larvae is soluble and consequently cannot hold the cells together (Herbst, 1900); Gray has shown that the cells of *Mytilus* tissue are bound together by a matrix the stability of which depends upon divalent cations. In view of such facts we can readily see that the action of Ca-free solutions on a complex tissue may be initially to loosen the matrix and thus increase the permeability to ions (or decrease the resistance), without affecting the cell membrane at all. Equally, in the later changes, as the resistance falls to the "lethal level" we cannot be sure that the observed decrease in resistance represents a progressive decline in the resistance of the membranes of all the cells. It may mean simply that different cells die at different times, whereupon their membrane resistance falls to zero, thus giving for a large number of cells, such as are found in a tissue membrane, an apparently steady decline of resistance which is simply due to a statistical effect. With regard to *b*, a similar difficulty exists: it is perfectly true that when divalent ions are added to or removed from the bathing solution there is an increase or decrease in resistance; this may or may not be due to a change in resistance of the cell membranes and, assuming it is actually due to the cell membranes, it does not follow from the change of resistance measured that the permeability of the membrane to ions has genuinely changed. The ions in the vicinity of the membrane are mainly an adsorbed population, adding e.g. Ca^{++} may simply change the ion population by displacing e.g. Na^+ . Then the bulky Ca^{++} ion may be much less able to penetrate the cell membrane than were the displaced Na^+ ions and so the resistance will rise. But this rise in resistance will not mean that the membrane has changed—it only means that the same membrane is less permeable to divalent than to univalent cations.

So we see once more that the detailed interpretation of resistance measurements is a very complicated matter. Similar difficulties have been found with observations on the red cell (Fricke & Curtis, 1934; Curtis, 1936). Fricke & Curtis concluded from their studies that the red cell swells up to the point of haemolysis without apparent change in resistance. After haemolysis or lysis by chemical lysins (saponin, complement and

amboceptor, adham, and cholate the membrane persists, but shows evidence of injury by a change in the frequency dependence of its capacity and resistance at low frequencies. It was concluded that "the injury cannot be due merely to a rupture in the membrane, but must be due to changes in the properties (increased permeability) of the membrane as a whole". Curtis subsequently revised this opinion: he subjected red cells to sublytic doses of lecithin, tannic acid, glucose, saponin, amboceptor and colloidal silicic acid, all of which should to some extent change the permeability of the cell membrane. Yet no change in the electrical properties of the red cells could be detected. It was concluded that this result "may mean that the form of the frequency variation is an extremely insensitive measure of permeability changes and capable of disclosing only the very great changes associated with haemolysis, or it may mean that the change in the frequency variation at low frequencies has nothing to do with permeability".

One authority on this field has remarked to us that electrical measurements are "the world's worst method of measuring permeability changes". It is certainly true that even the best techniques available at present cannot effectively detect changes of permeability which can, however, be observed by other methods. For example, Davson (1937) has shown quite clearly by chemical methods that, when subjected to osmotic swelling, the red cell membrane becomes much more permeable to K^+ at a stage of swelling much less than is necessary to cause haemolysis. But this change has not so far been detected by the electrical studies (Fricke & Curtis, 1935 a).

In spite of the disadvantage of lack of sensitivity, impedance studies have yielded a number of results of the highest physiological importance. Recently Curtis & Cole have studied the changes of impedance that accompany the passage of the impulse in squid nerve (1938 a, b) and *Nitella* cells (Cole & Curtis, 1938), finding that at the spike of the action potential the resistance of the cell membrane has fallen to about 1% or less of its resting value. Fig. 49 shows the time relationships of the monophasic action potential, and the membrane conductance which was measured simultaneously for *Nitella*. (The membrane conductance is inversely proportional to the membrane resistance.) The onset of the increase in conductance, in both nerve and *Nitella*, occurs somewhat after the start of the action potential, but coincides

quite closely with the point of inflection on the rising phase. "At this point the membrane current density reverses in direction, corresponding to a decrease of the membrane electromotive force, so that this E.M.F. and the conductance are closely associated properties of the membrane, and their sudden changes are themselves, or are due to, the activity which is responsible for the propagation of the nerve impulse. . . . Since the maximum observed conductances are still far from a complete permeability, and because the capacity changes are relatively slight (about 1

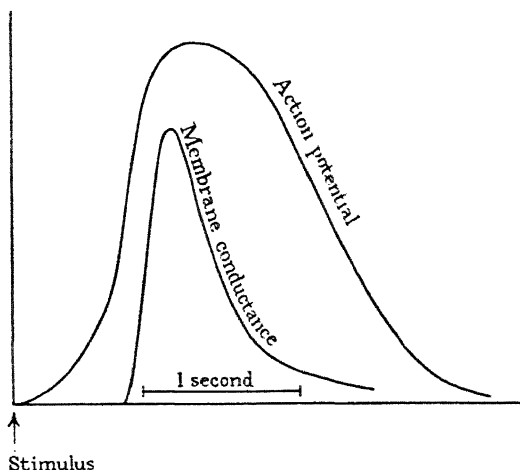


FIG. 49. *Nitella* membrane conductance, and monophasic action potential vs. time after stimulation (Cole & Curtis, 1938). Ordinates in arbitrary units. Frequency 0.2 kc.

decrease in squid nerve, 15% decrease in *Nitella*, we have indications that the excitation does not involve disintegration or destruction of the membrane." That this decrease in resistance of the cell membrane should accompany the passage of an impulse was predicted by R. S. Lillie (1923) on theoretical grounds; experimental difficulties for many years prevented its experimental detection and the eventual success of the impedance studies is one of the greatest technical achievements in the field of permeability. Blinks (1929) had previously shown that stimulation of *Nitella* produces a very large drop in resistance. A similar drop, from 250,000 to 10,000 ohms, is found when the bathing fluid (normally

pond water is replaced by 0.1 N KCl.* RbCl has a similar action, but with NaCl, LiCl, NH_4Cl , CsCl, CaCl_2 and MgSO_4 there is little change in resistance.

Dixon & Bennett-Clark (1929) have shown that the resistance of plant leaves decreases sharply on stimulation.

Other results have been obtained with *Arbacia* eggs, trout eggs and grasshopper eggs. According to Cole & Spencer (1938) a considerable change takes place in the membrane capacity of *Arbacia* eggs on fertilisation: the unfertilised egg has a membrane capacity of 0.86 microfarad per cm^2 and the fertilised egg 3.3 microfarads per cm^2 . This parallels a change in permeability to water and to ethylene glycol which takes place on fertilisation. On the other hand some marine eggs, e.g. those of *Cumingia* and *Chaetopterus*, show little change of membrane capacity on fertilisation (Cole & Curtis, 1938). Hubbard & Rothschild (1939) have shown that the impedance of trout eggs exhibits a peculiar rhythmical fluctuation which deserves further study. Cole & Jahn (1937) studied the changes in impedance with time of grasshopper eggs (*Melanoplus differentialis*). Before formation of the cuticulin the membrane has a resistance of 84 ohms per cm^2 and a capacity of 0.015 microfarad: after formation of the cuticulin the resistance rises to more than 10,000 ohms per cm^2 and the capacity to 0.1 microfarad.

Very many impedance studies have been made on muscle and on medullated nerve, but so far these have been extremely difficult to interpret owing to the complex nature of the tissues. Particularly important papers are those of Gildermeister, 1928; Lullies, 1930; Murali, 1935; and Cole, 1936. Rushton (1934) devised a method for measuring the resistance across the nerve sheath: this resistance proved to be mainly due to the medullating sheath and not to the excitable membrane (Cole, 1936), and is insensitive to changes in ionic composition and pH of the bathing fluid (Danielli,

* This drop in resistance does not necessarily involve a change in permeability. See the discussion on p. 208.

THE INTERPRETATION OF POTENTIAL DIFFERENCES ACROSS THIN POROUS MEMBRANES

The interpretation of potential measurements is a subject of great complication even in artificial systems where the constituent ions, whose diffusion gives rise to a potential, are known. In biological systems, where at present many of the ionic species are usually unknown, and where additional complications are introduced by variations in membrane permeability with time and by the dependence of potentials on cellular metabolism, the interpretation of potentials is not merely difficult, but frequently sheer speculation. Any conclusions on biological systems which are reached in the following pages are, therefore, at best provisional.

Let us consider first the potentials which may arise in artificial systems as a result of diffusion of ions in the absence of membranes. We may distinguish two simple cases of particular interest: 1, the potential arising when two solutions containing different electrolytes of the same concentration are brought into contact: 2, the potential arising when two solutions of the same electrolyte but of different concentration are brought into contact. In both cases the potentials are due to the fact that in one or both solutions the anion and the cation tend to diffuse at different speeds, so that the faster ion (e.g. H^+ in HCl solution) outstrips the slower ion (Cl^-): the resultant separation of electrical charges produces the measurable potential. The theory of these potentials was studied by Planck (1890) and Henderson (1907, 1908). Guggenheim (1933) gives an excellent and exact introduction to the thermodynamic theory of such potentials.

Case (1). If the two equimolar solutions contain univalent cations, e.g. Na^+ and K^+ respectively, and have a common anion, e.g. Cl^- , and the mobilities of these ions are U_{K_1} , U_{K_2} and U_A respectively, then the potential arising when the solutions are brought into contact is

$$E = 0.058 \log \frac{U_{K_1} + U_A}{U_{K_2} + U_A}. \quad \dots\dots (30)$$

Table XLIII gives values for such potentials for a number of different pairs of ions. When an ion of high mobility, such as H^+ , is paired against one of low mobility, such as Li^+ , a high potential is produced. Where the ions are of nearly the same mobility,

Na^+ and Li^+ , the potential is small. It will be seen that (30) is fairly accurate in dilute solution (0.01 M); it does not account for the variation in potential when the concentration is increased to 0.1 M . Lewis & Sargent (1909) introduced a semi-empirical correction for the effect of absolute concentration, and McInnes & Longworth (1936) give a graphical method for estimating such potentials which is accurate within the limits of experimental error.

TABLE XLIII. POTENTIALS IN MILLIVOLTS ARISING AT THE JUNCTION BETWEEN TWO EQUI-MOLAR SOLUTIONS OF DIFFERENT UNIVALENT ELECTROLYTES AFTER MCINNES & YEH

	0.01 M solutions	0.1 M solutions	Calculated (Henderson)
HCl: LiCl	33.8	34.9	33.8
KCl: NaCl	3.9	6.4	4.5
HCl: KCl	25.7	26.8	27.0
NaCl: LiCl	2.6		

TABLE XLIV. POTENTIALS IN MILLIVOLTS ARISING BETWEEN 0.1 M AND 0.01 M SOLUTIONS OF THE SAME ELECTROLYTE (AFTER MICHAELIS)

K_2SO_4	+ 15
KCl	0
LiCl	- 15
NaOH	- 32

Case 2. Again the potentials which arise are dependent upon different mobilities of the ions. If the two solutions are $N/10$ KCl and $N/100$ KCl, the potential produced is practically zero, because K^+ and Cl^- diffuse at practically the same speed, whereas with NaOH, which has one slowly moving ion (Na^+) and one fast ion (OH^-), a high potential is produced. Some typical examples are given in Table XLIV. The theoretical values for these three potentials are

$$E = 0.058 \log \frac{C_1}{C_2}, \quad \dots\dots(30.1)$$

where C_1 and C_2 are the concentrations in the more concentrated and more dilute solutions respectively. If the mobility of the cation is zero ($U_K = 0$), the formula reduces to

$$E = -0.058 \log \frac{C_1}{C_2}. \quad \dots\dots(30.2)$$

If the mobility of the anion is zero ($U_A = 0$), we have the same formula but with a change of sign:

$$E = +0.058 \log \frac{C_1}{C_2}, \quad \dots\dots 30.3$$

Effects due to Membranes. If instead of bringing the two pairs of salt solutions into direct contact we separate them by an inert porous membrane, the potentials arising should differ by very little from those found in the absence of the membrane. In practice it is found that membranes with very wide pores do conform to this condition, but that with most other membranes there is a very marked effect on the potential. The effect of the membrane is to change the relative mobility of the various ions by virtue of several different factors and this, as we can see from equations (28) and (29), must modify the potentials observed. Frequently the modifying influence of the membrane is so high as to reduce the mobility of all ions of one sign to zero. This is the case with dried collodion membranes, studied by Michaelis (1926) and many other workers. The normal dried collodion membrane is permeable only to cations. Table XLV shows the potentials arising when equimolar solutions of different electrolytes are separated by such a membrane. When the nature of the cation

TABLE XLV. POTENTIAL DIFFERENCES IN EQUIMOLAR SOLUTIONS SEPARATED BY A DRIED COLLODION MEMBRANE [AFTER MICHAELIS]

Salts with common anion Cl^-	Salts with common cat.
KCl : HCl $+93$	KCl : KBr
KCl : RbCl $+8$	KCl : KOH
KCl : KCl 0	KCl : K_2SO_4
KCl : NaCl -48	KCl : K^+
KCl : LiCl -74	KCl : $\text{K}_3\text{Fe(CN)}_6$

is changed, so is the potential. But changing the anion makes no difference to the potential. Not only is the mobility of the anion in such a membrane reduced almost to zero, but the relative mobility of the cations is also affected, so that the smaller cations diffuse at a greater rate, compared with the larger cations, than is the case in water. Thus the ratio of the mobilities of H^+ to Li^+ is about 10 in water and about 1000 in a dried collodion membrane.

One striking result of the impermeability to anions is that if a salt solution is separated from distilled water by such a membrane,

leakage of salt into the water is very slow. But if two solutions having different cations are used, exchange of the cations occurs rapidly, the anions of course showing little or no exchange.

The same impermeability to anions is shown when two solutions containing the same salt in different concentrations are separated by a dried collodion membrane. The mobility of the anion is nil, i.e. $U_1 = 0$, so that for a concentration difference of tenfold the potential should be 58 millivolts [equation (29)]; this value is very nearly reached with $N/10$ and $N/100$ KCl separated by a dried membrane. There are, however, very marked effects found when the value of C_1/C_2 is constant, but the absolute concentrations are varied. This concentration effect cannot be accounted for by the simple theory given above.

Mond & Hoffman (1928) and Wilbrandt (1935) have studied collodion membranes containing basic substances, such as rhodamin B. Mond found that when collodion membranes are prepared containing suitable bases, an anion-permeable membrane is obtained instead of the usual cation-permeable type. Table XLVI shows the potentials obtained with such a membrane. The behaviour is exactly the opposite of that of the cation-permeable membranes; the potential is greatly affected by the nature of the anion, but not at all by the nature of the cation.

TABLE XLVI. POTENTIAL DIFFERENCES IN MILLIVOLTS ACROSS A DRIED COLLODION MEMBRANE STAINED WITH RHODAMIN B. 0.1M SOLUTIONS AFTER MOND

NaCl : NaSCN	-60
NaCl : NaNO ₃	-51
NaCl : NaI	-33
NaCl : NaBr	-20
NaCl : NaCl	0

Membranes of amphoteric materials, such as gelatin, are predominantly permeable to cations on the alkaline side of the iso-electric point, to anions on the acid side, and at the iso-electric point itself exert preferential action on the mobility of neither cation nor anion.

Michaelis suggested that the reason why collodion membranes were normally cation permeable is that the membrane consists of very narrow pores, on the walls of which anions such as Cl^- were adsorbed. Then, if the pore is sufficiently narrow, no more

anions will be able to enter owing to the electrostatic repulsive action of the adsorbed anions: only cations will be free to diffuse through the pores and the membrane will therefore be exclusively cation permeable, since the adsorbed anions are fixed on the walls of the pores and so cannot migrate. Northrop (1929) and Beutner *et al.* (1933, 1934) have regarded the matter as more a question of the partition coefficients of the different ions between collodion and water.

There is in any case great difficulty in accepting the idea that the anions are immobilised by adsorption on the walls of the collodion pores. The polar character of collodion is little calculated to promote the adsorption of anions and in addition there are already anions (SO_3^- and CO_2^-) attached to the structure of the nitrated polysaccharide chains of which collodion is composed. With these facts in mind, Wilbrandt (1935) developed a view which to some extent resolved the conflict between the views of Michaelis and of Northrop, and which has furnished the basic ideas for a more accurate quantitative treatment of the potentials.

Wilbrandt pointed out that there is agreement among all workers that the more permeable collodion membranes have a porous structure. Collodion membranes are prepared by dissolving collodion in an organic solvent, forming a thin layer of the solution and then allowing the solvent to evaporate. If, before the whole of the organic solvent has evaporated, the membrane is plunged into water, a comparatively permeable membrane is obtained, having a large average pore diameter. But if the whole of the solvent is allowed to dry out, the membrane is highly impermeable. Northrop suggested that in these latter membranes the pores have disappeared, and that the membrane allows molecules to penetrate only if they are soluble in the substance of the membrane. Wilbrandt argued that "according to this assumption, the membrane would change its behaviour decisively, when losing the last part of the organic solvent. One would expect that this more or less sudden change would also appear in its electromotive behaviour, in the form of a discontinuity in the plot of potential against permeability. This, however, is not the case (as Fig. 50 shows). The transition from membranes with large pores to those with supposedly no pores is steady". It is concluded that the transition from the very permeable, or "wet", to the impermeable, or "dried", membranes consists of a gradual

transition of pores into intermolecular spaces, the change thus being only quantitative, not qualitative.

The question then arises, what is the character of these intermolecular spaces? To begin with, they will be of two sorts, since X-ray examinations of dried collodion membranes have shown

Mathieu, 1933 that their structure is built up on a basis of random arrangements of microcrystals of nitrocellulose. There

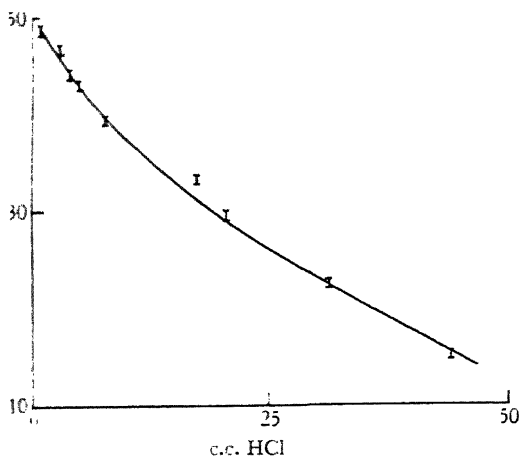


FIG. 50. Relationship between permeability to ions and E.M.F. across collodion membranes of varying porosity. E.M.F. in the system $0.1M$ KCl:membrane: $0.01M$ KCl plotted against equivalents of HCl diffusing across the membrane in 8 hours, in the system HCl:membrane:water (modified from

will thus be (1) spaces between the micro-crystals which will be of one character, and (2) spaces between adjacent molecules in the micro-crystals which will be of rather different character. Some substances will be able to penetrate only those spaces between the micro-crystals and others will be able to penetrate also the crystal lattice of the nitrocellulose. It seems a reasonable hypothesis that ions will fall into the former group of substances and that very small molecules like hydrogen, or solvents for nitrocellulose such as butyl alcohol, will be able to penetrate the lattice also. Thus the collodion membrane resembles membranes of silica glass, which is also composed of minute crystals: the larger gas

molecules, such as nitrogen and argon, can penetrate between the crystals only, but the smaller molecules, hydrogen and helium, can also penetrate the crystal lattice.

Evidently, in dealing with ions, the spaces between crystallites will be of predominant importance. The disposition of the electrical charges of the molecular dipoles of a nitrocellulose chain is such as to give the surface of these interspaces a predominantly negative charge. To this effect must be added the action of the negative charges of SO_3^- and CO_2^- groups present. "An adsorption of anions on these surfaces is not to be expected. On the other hand the negative charge of the surfaces may be proven, not only by the sign of the concentration potential, but also by the direction of electro-osmosis across the membrane, or cataphoresis of collodion particles. The electric field, which counteracts the entrance of anions into the pore, is established... by the membrane itself.... So within the total area of the pore there will be a certain fraction, due to (the repulsive action of this field ...into which anions cannot enter, while cations can. The extent to which, due to this situation, the apparent average mobility of anions in the pore is decreased relatively to the cation mobility, therefore depends on the ratio of this fractional area to the total area of the pore. Hence the concentration potential depends on the size of the pore" (Wilbrandt, 1935).

Utilising such ideas as these brought forward by Wilbrandt, Teorell (1935) and Meyer & Sievers (1936) have been able to put certain aspects of the problem of potentials developed across porous membranes into quantitative form. This involves treating the membrane as an electrolyte with certain ions (those fixed to the membrane structure) having zero mobility. Meyer & Sievers have also considered (1) the fact that pores of less than a certain diameter will admit certain ions but not others (called by them the "sieve effect") and also (2) that the different ions may have different partition coefficients between membrane and water, due to the different Van der Waals' forces, etc. of the ions. Let us consider a simple case of the potential arising when two solutions of NaCl, concentrations C_1 and C_2 , are separated by a membrane containing a concentration A of immobile anions. Then, when ions have diffused until the steady state has been reached, the distribution of ions in the membrane will be that shown by Fig. 51. Since there are fixed ions in the membrane, there will be Donnan

potentials E_1 and E_2 in the membrane to be considered (see Chapter III) arising at the boundary between the membrane and solutions 1 and 2. So that the total membrane potential will be

$$E = E_1 + E_2 + \Pi,$$

where Π is the diffusion potential in the interior of the membrane; all these potentials can be calculated. Teorell gives the results shown in Table XLVII for the case in which $A = 1$, $C_1 = 100$, and C_2 is varied. As C_2 is diminished the net potential E becomes negative, reaches a limit and then in still more dilute solutions becomes positive again. This type of behaviour has been observed with frog skin by several observers.

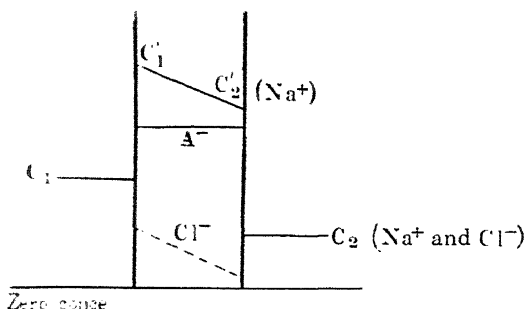


FIG. 51. Distribution in the steady state of ions in a membrane separating two solutions of NaCl, concentrations C_1 and C_2 . The membrane contains a number of immobile anions.

TABLE XLVII. POTENTIAL BETWEEN TWO NaCl SOLUTIONS OF CONCENTRATIONS $C_1 = 100$, C_2 VARIABLE, SEPARATED BY A MEMBRANE FOR WHICH $A = 1$, MOBILITY RATIO Na : Cl = 0.63 (TEORELL)

C_2	Donnan term $E_1 + E_2$	Diffusion term Π	Total potential difference
100	0	0	0
10	1.2	-13.2	-12
5	2.2	-17.4	-15.2
1	12.2	-26.4	-14.2
0.5	22.2	-29.4	-7.2
0.1	58.0	-31.6	+26.4

We may say in conclusion that the theory of diffusion of ions across a porous membrane has reached a very promising stage and it is just possible that bio-electric potentials are to some

extent similar to those across porous membranes. A good deal more work is necessary, however, before the precise significance of certain of the constants in the equations of Teorell and Meyer & Sievers can be determined.

Other difficulties in the application of such theories to biological tissues have been raised by Keys (1937): "A major difference between artificial membranes and the more important animal membranes is in the thickness. Collodion membranes are seldom less than 100μ (0.01 cm.) in thickness....The membranes of the capillary wall, the alveolus of the lung, the villi of the intestine, etc. are so much thinner—of the order of at most a few microns—that it seems doubtful whether they are comparable. Certainly Teorell's postulates cannot apply to the plasma membranes of cells, which are probably at most a few molecules in thickness. Furthermore most of the animal membranes which may be thick enough to come within Teorell's scheme have the complication that the thickness is made up of living, i.e. metabolising, cell substance."

With regard to the last of these points, we can say with assurance that in so far as metabolism contributes to the potentials measured across frog skin, for example, the potential due to metabolism is an additional factor, to be added (not necessarily algebraically) to the potentials predicted by the theory of Wilbrandt, Teorell and Meyer & Sievers. It is quite certain that the latter potentials will exist across such tissues, and the problem as to how far the observed potentials are due to metabolism and how far to simple physical processes remains to be solved. In the case of comparatively porous systems, such as the capillary membrane, the thickness may be raised to the order of a micron due to the unstirred layer of water adhering to the walls of the capillary, as may be seen by microscopical examination.* Teorell (1937) believes that when this unstirred layer is considered, a layer of sufficient thickness is provided for the diffusion potentials to develop. In so far as this is true, the water layer itself must contribute to the membrane potential in a way characteristic of *water*, not of the membrane. Thus the observed potential will fall between a membrane potential and an ordinary liquid junction potential such as is found in the absence of a membrane.

* Such observations must be utilised with caution, since it is known that there is a rapid flow of fluid through the capillary pores.

With regard to the plasma membrane, Teorell (1937) thinks that "the concept of the plasma membrane being only a few molecules thick is more of 'morphological' significance than of functional: if the actual membrane consisted only of a monolayer, its 'effective' thickness in regard to the effect on diffusion across it is very much greater owing to the presence of unstirred layers of the surrounding solutions, one on each side of the membrane. These 'unstirred layers' are bound to exist due to pure hydrodynamical reasons. Working on plain, very thin cellophane membranes, I have found that the average thickness of the unstirred layer was around 30μ even at very vigorous stirring of the bulk solutions. Dr Schulman and I have recently found that there exists a rigid layer of the aqueous solution underneath monomolecular films, of the order of $20\text{--}30\mu$. These observations suggest that the effective thickness of biological diffusion layers can hardly be less than a few microns." With this view the present writers are in fundamental disagreement. We quite agree that under certain conditions there is an unstirred layer of the order of $20\text{--}30\mu$. This layer, however, cannot be regarded as rigid: in fact we know of many instances in biological systems where vigorous flow occurs in a layer only $5\text{--}10\mu$ in diameter, e.g. cyclosis in plant cells, blood flow in capillaries, so that it is very doubtful whether the existence of unstirred layers greater than $2\text{--}3\mu$ in thickness are ever necessary to normal membrane function in biological systems. In the case of the plasma membrane, which has a thickness of the order of 10^{-6} cm., we have already seen that a resistance is offered to the free diffusion of most molecules (Chapter VIII) which is of the order of 10^9 times greater than that of an equal thickness of water; e.g. the plasma membrane offers a resistance to free diffusion of the order of that due to 10^3 cm. of water, so that it is hardly to be supposed that an addition of a few μ of water will make much difference to the observed resistance. We regard the high resistance to free diffusion, offered by the plasma membrane in a thickness of the order of 10^{-6} cm., as one of the most fundamental properties of the plasma membrane, one representing a highly efficient adaptation of membrane properties to the function of maintaining the normal interior composition of the cell. That the resistance to free diffusion is located in such a thin layer is probably also essential for the phenomena of excitability and conduction of electrical impulses.

THE INTERPRETATION OF POTENTIAL DIFFERENCES ACROSS OIL LAYERS

When two aqueous salt solutions of different concentration or different composition are separated by an oil layer, a potential difference may be detected across the oil layer. These potential differences have been studied intensively by Beutner *et al.* 1913-1931 and by Baur *et al.* 1913-1923.

If a neutral oil such as paraffin is used, the potentials with inorganic salts are very low. If, however, the oil is acidic in character, e.g. contains a phenol or a fatty acid, a substantial potential difference can be found. Table XLVIII shows potentials obtained by Loeb & Beutner (1913) for the system:

saturated KCl guaiacol + oleic acid KCl, variable concentration.

The oil layer behaves as a preferentially cation-permeable membrane. If, on the other hand, the oil is basic, containing e.g. toluidine, it behaves as a preferentially anion-permeable membrane. Extensive studies have also been made on the system: $0.1\text{ }M\text{ NaCl/oil/}0.1\text{ }MX$, where X is an electrolyte other than NaCl. For example, if the oil is cresol and X = sodium oleate, the potential is 100 millivolts; if X = aniline hydrochloride, the potential is reversed in sign, and equal to 50 millivolts. Beutner and his colleagues have shown that there is a correlation between the staining reaction of oils and the nature of the potentials developed across them; "cation-permeable" oils are preferentially stained by basic dyes, and "anion-permeable" oils by acid dyes. The correlation extends to a parallelism between the intensity of staining and the magnitude of the potentials developed.

TABLE XLVIII. CHANGES OF POTENTIAL WITH VARIATION OF KCl CONCENTRATION IN THE SYSTEM SAT. KCl GUAIACOL + OLEIC ACID KCl VARIABLE CONCENTRATION

	Millivolts
Changing from 0.5 <i>M</i> to 0.1 <i>M</i>	28
" " 0.1 <i>M</i> to 0.02 <i>M</i>	28
" " 0.02 <i>M</i> to 0.004 <i>M</i>	32

All the measurements mentioned above were made with layers of oil several centimetres thick. Danielli (1936) devised a method of forming thin films of oil, which in the presence of proteins are stable for days. The technique is similar to that of blowing bubbles

in air, with the soap solution replaced by oil and the air replaced by water (Fig. 52). These films are much thinner than any oil layers previously used, but are still much thicker than cell membranes. K.S.Cole personal communication, from some preliminary impedance measurements, has concluded that such membranes when formed of triolein are several hundred molecules thick. The chief difficulties in making thinner films are the high viscosity found with sufficiently insoluble oils, and the fragility of such films when large in area. Fragility sets a limit to thinning by blowing the bubble larger, and thinning by draining is an

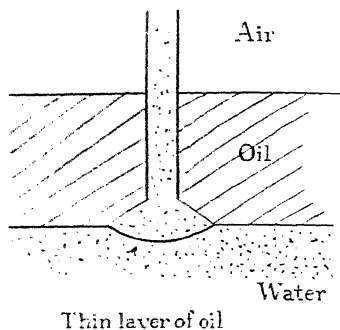


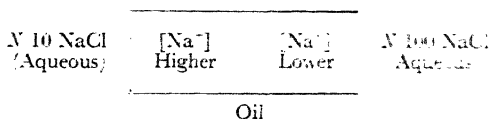
FIG. 52. Method of making a thin oil film between two aqueous layers (Danielli).

extremely slow process with a viscous oil when the thickness falls below 1μ , owing to the high viscous resistance to flow, which increases roughly as the inverse third power of the thickness of the film.

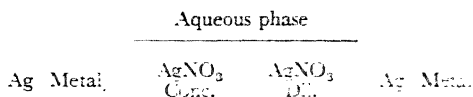
Wilbrandt (1935) has studied such films and has found that in addition to the usual potentials there are some curious transitory changes in potential, which appear to be due to the spontaneous breakdown of a whole oriented monolayer, which sweeps away the ionic atmosphere at the oil-water interface. This observation has been confirmed by Teorell (1936). Dean & Gatty (1940), using a similar technique, but with oils of much lower viscosity and specific conductivity, have found many interesting phenomena, which may or may not be very closely related to biological problems. It is very doubtful whether results will be obtained

which we may say with confidence are analogous to the bio-electric potentials of cell membranes until much thinner films are used, made of oils of specific resistance between 10^{10} and 10^{12} ohms, and of a viscosity 10^2 – 10^5 times that of water.

Beutner considered that these potentials are phase-boundary potentials, due to the variation in partition coefficient of various ions between the oil phase and the water phase. For example, in the system: 0.1*N* NaCl oleic acid 0.01*N* NaCl, since there is no organic cation in the oil phase, the oil-water partition coefficient of Cl^- is very low, and its concentration in the oil phase may be neglected (to a first approximation). But, owing to the fat soluble anions supplied by the oleic acid, Na^+ has a significant solubility in the oil layer. Consequently the cell may be represented according to Beutner) as



and this may be compared to the system



i.e. the NaCl solution may be regarded as similar to Ag electrodes, and the phase-boundary between the NaCl solutions and oil "produces electromotive actions similar to the phase-boundary between Ag metal and the aqueous AgNO_3 solution....An essential similarity of these systems is that both have a transitional layer in the centre between the two solutions of different concentration" (Beutner, 1933*b*, p. 221). To the present writers this appears to be an argument essentially by analogy, and that this analogy is far from complete: for example, the NaCl solutions are compared with Ag electrodes. But the ion concentrations in the Ag electrodes are the same on both sides of the cell, whereas it is an essential point in the oil system that the ion concentrations in the (according to Beutner) comparable NaCl solutions shall be different. So, whilst agreeing with Beutner that the potentials

observed do include a term of major importance due to the uneven partition of ions across the oil-water interfaces, we prefer to discard the argument by analogy, and, in so far as it is possible, deal only with calculations of the direction and magnitude of the potential which are derived from the actual dispositions i.e. concentration and mobility of the individual ions.

Baur for long considered that the potentials were adsorption potentials, finding their origin in the adsorption of charged molecules and ions at the oil-water interfaces. Such potentials are well known at the air-water interface [see Adam, 1938] and may be of considerable magnitude. Potential changes are known

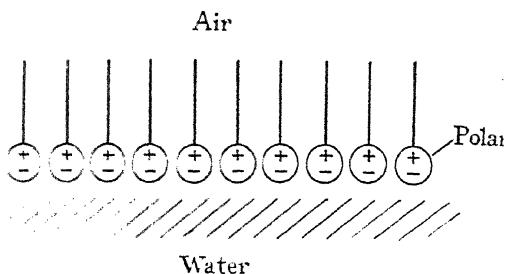


FIG. 53. Oriented charged molecules at an air-water interface.

of over a volt due to the spreading of a monolayer at an air-water interface. These potentials are due to the fact that the molecules carry fixed positive and negative charges; when in solution the molecules, and therefore the charges, are oriented at random and so cannot give rise to potentials; but when the molecules are adsorbed in a monolayer the orientation is to some extent fixed, so that the charges are arranged in a fairly definite way, and a potential results [Fig. 53]. Although Baur finally discarded this view, and adopted an attitude essentially similar to that of Beutner, his original view has not been altogether discarded by other workers. Recently Dean *et al.* (1940), on theoretical grounds, predicted that adsorption potentials at the oil-water interface could only be transitory, for around an oriented dipole such as $C^+=O^-$ will collect an atmosphere of ions, predominantly negative ions around the positively charged carbon atom, and

predominantly positive ions round the negatively charged oxygen atom, so reducing the net orientation of charge to zero. It takes some time for the potential due to a suddenly formed adsorbed layer to fall to zero, because the ions required round the positive end of the dipole have to collect in the oil layer: it is difficult for ions to pass from water into the oil, and consequently the potential falls off slowly.* Dean & Gatty (1940) have shown experimentally, by spreading monolayers at the oil-water interface, that whilst the potential due to such an adsorbed layer is transitory, it does not necessarily fall off too rapidly to be measured.

We may consequently conclude that adsorption potentials have little to do with the semi-permanent potentials observed across thick layers of oil. It would probably be inadvisable to discard consideration of adsorption potentials entirely, when considering biological systems, since in such systems we are dealing with non-equilibrium, i.e. more or less transitory states, almost exclusively.

K. H. Meyer (1936) has suggested that the same essential treatment may be applied to oil membranes as to collodion membranes (see p. 219). This seems the most encouraging of the suggestions put forward for detailed treatment of potentials across oil layers. It is suggested that an oil layer may be regarded as essentially similar to solid membranes, except in the one particular that being liquid the oil layer has no permanent structure. Consequently (1) there can be no fixed ions, incapable of diffusing: 2 there can be no sieve effect. Hence the different actions of the different ions must be due to differences (*a*) in their partition coefficients, and (*b*) in their mobilities.

Now consider the case of an oil layer separating two concentrations C_1 and C_2 of the same univalent salt in aqueous solution. Let l_A and l_K be the partition coefficients of the anion and cation respectively. Then, when the steady state has been reached, the concentration of salt in the oil immediately adjacent to the aqueous phase of concentration C_1 will be $C_1 \sqrt{l_A l_K}$, and immediately adjacent to the other side will be $C_2 \sqrt{l_A l_K}$. Owing to these distributions of ions we obtain three potentials, as in the case of a solid membrane, one at each oil-water interface, and

* At the air-water interface there is insufficient depth of hydrocarbon liquid behind the adsorbed molecules for the formation of a complete ionic atmosphere; consequently the potentials do not fall to zero.

a Henderson diffusion potential between the two concentrations of ions in the oil phase. Hence the observed potential is

$$\begin{aligned}
 E &= E_1 - II - E_2 \\
 &= \left[\frac{RT}{F} \log_e \sqrt{\frac{\bar{l}_K}{\bar{l}_A}} \right] + \left[\frac{RT}{F} \frac{U_K - U_A}{U_K + U_A} \log_e \frac{C_1 \sqrt{\bar{l}_A \bar{l}_K}}{C_2 \sqrt{\bar{l}_A \bar{l}_K}} \right] \\
 &\quad - \left[\frac{RT}{F} \log_e \sqrt{\frac{\bar{l}_K}{\bar{l}_A}} \right] \\
 &= \frac{RT}{F} \frac{U_K - U_A}{U_K + U_A} \log_e \frac{C_1}{C_2}, \quad \dots\dots 30.4
 \end{aligned}$$

where U_K and U_A are the mobilities of the cation and anion in the oil phase.

Similarly, for two different univalent salts I and II of the same concentration the potential is

$$\begin{aligned}
 E &= E_1 - II - E_2 \\
 &= \left[\frac{RT}{F} \log_e \sqrt{\frac{\bar{l}_{K_1}}{\bar{l}_{A_1}}} \right] \\
 &\quad - \left[\frac{RT}{F} \frac{U_{K_1} - U_{A_1}}{U_{K_1} + U_{A_1}} - \frac{U_{K_2} - U_{A_2}}{U_{K_2} + U_{A_2}} \right] \log_e \frac{U_{K_1} + U_{A_1}}{U_{K_2} + U_{A_2}} \\
 &\quad - \frac{RT}{F} \log_e \sqrt{\frac{\bar{l}_{K_2}}{\bar{l}_{A_2}}}, \quad \dots\dots 30.5
 \end{aligned}$$

where \bar{l}_{K_1} = partition coefficient of cation of salt I, \bar{l}_{K_2} = partition coefficient of cation of salt II, etc. When the two salts are identical the equation 30.5 of course reduces to equation (30.4). Meyer concludes that the permeability of an oil layer to an ion depends on (a) its mobility, (b) its partition coefficient, (c) the mobilities and partition coefficients of the other ions present. The differences in mobility and solubility of ions result from an interaction between the ions and the solvent, and in a liquid membrane there cannot be an ionic selectivity or sieve effect such as is found in membranes possessing structure.

The application of the theory of Wilbrandt, Teorell and Meyer & Sievers to biological potentials. From the detailed treatment of Meyer & Sievers we may distinguish a number of special cases of particular interest to the experimentalist.

1. The same salt is kept on both sides of the membrane, the absolute concentration of the salt on the two sides is varied and the ratio C_1/C_2 is kept constant. Then if the potential difference is constant, according to Meyer & Sievers $A=0$, i.e. there are no fixed anions and cations, the pore size does not change with dilution, and the partition coefficients of the ions do not change with dilution (or else the net effect of these variables is to cancel out).

2. If in case (1) certain cations give rise to much higher potentials than others, or certain anions are more effective than others, then either the sieve effect, the partition coefficients or the mobilities are responsible for the observed differences.

3. If the potential difference is very dependent on the absolute values of C_1 and C_2 , when C_1/C_2 is kept constant, then either the membrane contains fixed ions ($A \neq 0$), and/or the pore size and/or the partition coefficient of one or more ions is greatly affected by concentration.

Whilst these and perhaps other special cases present features of great interest in the biological field, we doubt whether it is possible in the case of the cell membrane to arrive at a more satisfactory conclusion than that the potentials observed are due to one or more of several variables, without considerably more knowledge of the structure of the cell membrane than is at present available. In analysing the behaviour of an artificial membrane by the method of Meyer & Sievers it is possible to draw upon large amounts of information from chemical and X-ray sources. Where the cell membrane is concerned such information is only just appearing on the scene. A further difficulty may arise from the dimensions of the cell membrane. It is an essential feature of the theory of Meyer & Sievers that diffusion across the membrane interface shall be rapid compared with diffusion across the interior of the membrane, since unless this is so the concentration of ions in the membrane adjacent to a solution of concentration C will not attain its equilibrium value, $C \sqrt{l_A l_F}$. But, as is shown in Appendix A, the rate of diffusion across such an interface is, for the cell membrane, frequently much less than the rate of diffusion across the interior of the membrane. Applications of the views of Meyer & Sievers to the cell membrane must, therefore, be at best provisional.

Surveying the results of experience with porous membranes and with oil membranes, it must be confessed that we are still but

touching on the fringes of the knowledge, both theoretical and experimental, which is necessary for the interpretation of cell membrane potential.

BIOELECTRIC POTENTIALS

Most biological membranes under normal conditions have a potential across them, due to the uneven distribution of various ions which is characteristic of living systems. Sometimes these potentials are fortuitous, being a form of waste product of vital activity; sometimes the potentials are known to serve a useful purpose. In both cases information may be obtained about the permeability of the membranes concerned, by observing the natural potential, and by causing this potential to change by means of a carefully controlled modification of the ionic composition on one or both sides of the membrane.

Studies have at present been mainly on nerve, muscle, large plant cells and frog skin. A considerable amount of work has also been done on such membranes as the cuticle of apples (Loeb & Beutner, 1913; Fujita, 1925; Michaelis, 1926) which is of more historical than immediate interest.

Let us first consider nerve and muscle. If contact at two different uninjured points is made on a resting nerve or muscle, no potential difference can, as a general rule, be observed.* When the tissue underlying one electrode is damaged, a considerable potential difference is found between the two points, such that the injured region is negative relative to the normal region. This is known as the injury, or resting potential. It is generally considered that, as a result of the injury, the electrode touching the injured region is in contact with the intracellular fluid, and therefore indirectly with the interior surface of the cell membrane, whereas the electrode touching the normal region is in contact with the exterior of the cell membrane. The variation in this potential when the exterior fluid is changed has been extensively studied, especially by Macdonald, Höber, Cowan and Wilbrandt. Macdonald (1905) used NaCl, KCl, HCl and NaOH solutions, observing their influence on the resting potential of medullated nerve. His results are complicated by osmotic effects due to

* This is not true of large plant cells, in which local regions may differ in membrane potential by as much as 30 millivolts. (Osterhout).

failure to use isosmotic solutions, but nevertheless it was shown that the resting potential is not much affected by variation in NaCl concentration and is approximately a linear function of the logarithm of the K^+ concentration in the bathing fluid: i.e. we can write, according to Macdonald,

$$E = 0.058 \log \frac{C_o}{C_i}, \quad \dots 30.3$$

where C_o is the concentration of K^+ in the outside fluid, and C_i is the apparent concentration, presumed constant, of K^+ in the interior of the nerve fibres. This, as we have seen above, is the equation for the diffusion potential between two KCl solutions separated by a membrane permeable only to cations, but in this case the membrane is even more selective, being practically impermeable to all cations other than K^+ . Consequently Macdonald concluded that the resting potential of nerve is a diffusion potential, and that the nerve membrane across which this potential arises is non-aqueous and exclusively permeable by K^+ . It must be pointed out, however, that these conclusions were hardly justified, and in particular that the potentials, although a linear function of $\log C_o$, never reached the value demanded by the equation $E = 0.058 \log C_o/C_i$. This has generally been regarded as due to short-circuiting of the potential difference by the inter-cellular tissue spaces, but adequate proof of this has never been obtained.

Cowan (1934) obtained essentially similar results with unmyelinated nerve of *Maia*. He used solutions isosmotic with sea water and varied the K^+ content, finding that the observed E.M.F. was, within experimental error, a linear function of $\log C_o$ (Fig. 54). If the theory of Meyer can be applied to this membrane, it would follow that $A = 0$, i.e. that there are no fixed ions in the membrane and that there is either a pronounced sieve effect, partition effect or effect due to exceptional mobility of K^+ in the membrane. Cowan found that the order of effectiveness in reducing the resting potential was $K^+ > Rb^+ > Cs^+$. It was also shown that potassium leaks out of the nerve as a result of conducting an impulse, and that the amount leaking out is roughly proportional to the number of impulses conducted. This is clear evidence of an increased permeability to K^+ during, or as a result of, the passage of an impulse. Asphyxiation decreases the

IMPEDANCE AND POTENTIAL MEASUREMENTS

potential in a reversible manner, and this is interpreted to mean that the permeability to ions changes. The decrease might be due to an increased permeability to other ions, or to a decreased permeability to K^+ : the latter possibility is far more probable. When *Maia* nerves are stimulated to fatigue, or asphyxiated for too long, large amounts of K^+ escape from the fibres. Wilbrandt has shown that on stretching *Maia* nerve the potential falls off, but when the nerve is restored to its original length the potential is restored also (personal communication).

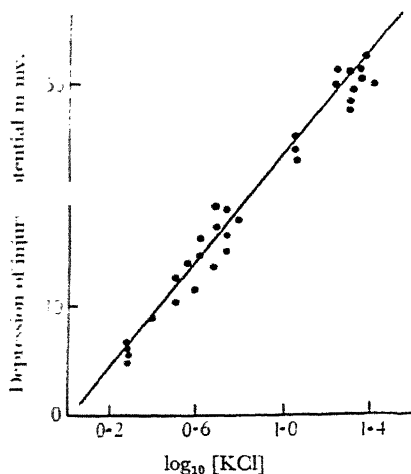
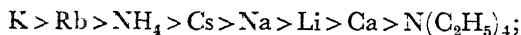


FIG. 54. The effect of variation in the potassium content of the fluid bathing *Maia* nerve on the resting potential. The fluids were all isosmotic with sea water.

Other workers have investigated other ions. According to Netter (1927), cations influence the resting potential of frog nerve medullated in the following order:



whereas anions have little or no action. Höber & Ströhe (1929) reached much the same conclusion. Wilbrandt (1937) studied the non-medullated nerves of *Maia*, obtaining the series $Rb > K > Na = Li$, which agrees with Netter's results on frog nerve, except in the case of Rb. Anions were also shown to have a small effect on the potential, in the order $SCN > NO_3 = Br = Cl$. The

influence of SCN is about equal to that of Na , and that of Cl a little less. The dialkylamines $R_2\text{NH}$, where $R = \text{C}_n\text{H}_{2n+1}$, showed two very clear-cut effects as the size of the alkyl residue was increased. From dimethylamine to dipropylamine the potential changes very little, much less than it would with the same concentration of K^+ , but a little more than with Na^+ . The activity falls off slightly on passing from dimethyl to dipropyl. But on passing to dibutylamine, and still more with diamylamine, there is a great increase in activity: the latter compound has an activity comparable with K^+ . These latter two amines are not strictly reversible in their action within a reasonably short time. Somewhat similar results were obtained with medullated frog nerve, except that the reversing point is a little different: dimethyl and diethylamine have appreciable activity, dipropyl and dibutylamine have little more activity than has a non-electrolyte such as glucose, whereas diamylamine $\text{C}_5\text{H}_{11}\text{NH}_2$ has a very marked activity. The final series of activities in affecting the potential was $\text{Li} = \text{Na} = \text{choline} = \text{tetramethylamine} = \text{dipropylamine} < \text{dimethylamine} = \text{diethylamine} < \text{tetraethylamine} < \text{guanidine} < \text{dibutylamine} < \text{diamylamine} = \text{K} < \text{Rb}$.

Table XLIX, taken from Höber (1937), summarises the results obtained with various organic substances on both medullated and non-medullated nerve. It is notable that all the substances which are active on frog nerve are also active on crab nerve, but that medullation apparently shields the frog nerve from a number of substances which are active on crab nerve.

The conclusions as to the action of ions on the resting potential of nerve are in general similar to those reached on muscle. Höber (1905) obtained the following series for the activity of different ions in changing the resting potential of frog muscle:

Cations $\text{K} > \text{Rb} > \text{NH}_4 > \text{Ca} > \text{Mg} > \text{Na} > \text{Li}$;

Anions $\text{SO}_4 > \text{HPO}_4 > \text{acetate} > \text{Cl} > \text{B} > \text{I} > \text{NO}_3 > \text{CNS}$.

Table L summarises results obtained with organic substances on muscle. The results are essentially similar to those obtained with crab nerve. With both crab nerve and muscle the observed resting potentials are less than would be expected for a true potassium concentration potential. For *Maia* nerve, the calculated potential in Ringer is 66 millivolts, the observed potential about 30 millivolts; for frog muscle the calculated and observed values are 73 and

TABLE XLIX. ACTION OF VARIOUS SUBSTANCES UPON THE RESTING POTENTIAL OF NERVE

+ means an observable diminution of the resting potential at the concentration given

Cation	Frog nerve medullated			Crab nerve non-medullated		
	Effect	pH	Molar	Effect	pH	Molar
Acetylcholine	+	7	.	+	8	1/100000
Dipropylamine	0	7	1/13	0	8	1/2.5
Dibutylamine	0	7	1/13	+	8	1/10
Diamylamine	+	7	1/13	+	8	1/10
Dicaprylamine	+	7	1/600	.	.	.
Veratrine	+	7	1/10000	+	8	1/50000
Strychnine	0	7	1/200	0	8	1/180
Codeine	0	7	1/200	0	8	1/32
Novocaine	0	7	1/50	0	8	1/34
Non-electrolytes						
Ethylurethane	+	7
Amylurethane	+	7	1/26	.	.	.
Anions						
Valerate	0	7	1/20	+	7	1/20
Caprylate	0	7	1/40	+	8	1/10
Nonylate	0	7	1/40	.	.	.
Caprate	.	.	.	+	8	1/20
Laurate	.	.	.	+	7	1/200
Oleate	+	7	.	+	8	1/200
Cholecholate	.	.	.	+	8	1/250
Taurocholate	0	7	1/160	.	.	.
Salicylate	0	6.5	1/40	+	.	1/20
Saponin	0	7	0.5%	+	8	0.5%
Free Acids						
Caprylic acid	+	4	1/250	.	.	.
Hydrochloric acid	0	3.5	1/10000	.	.	.

TABLE L. ACTION OF VARIOUS SUBSTANCES IN THE RESTING POTENTIAL OF FROG MUSCLE

+ means an observable diminution of the resting potential at the concentration given

Cations	Effect	pH	Molar	Anions	Effect	pH	Molar
Diethylamine	0	7	1/100	Acetate	0	7.5	1/60
Dipropylamine	0	7	1/100	Butyrate	0	7.5	1/160
Dibutylamine	0	7	1/100	Lactate	0	7.5	1/160
Diamylamine	+	7	1/500	Capronate	+	7	1/40
Dicaprylamine	+	7	1/5100	Caprylate	+	7	1/160
Acetylcholine	+	7	1/100000	Nonylate	+	7	1/5100
Codeine	+	7	1/40	Laurate	+	7	1/1300
Atropine	+	7	1/2800	Oleate	+	7	1/1300
Veratrine	+	6.4	1/1000	Salicylate	+	7	1/1300
				Taurocholate	+	7	1/320
Non-electrolytes							
Hepylate & E	+	7	satur.				
Butylurethane	+	7	1/40				
Amylurethane	+	7	1/52				

42 millivolts respectively (Cowan, 1937). As in the case of frog nerve, the low value of the resting potential is usually attributed to short-circuiting.

So far as nerve and muscle are concerned, then, we may say that the cell membrane has a preferential permeability to certain inorganic cations, but is also permeable to anions, though (apparently) to a much smaller extent. Moreover, amongst the cations themselves there are marked differences, Li^+ being a little more effective in penetration than an anion, and K^+ having very great activity. Amongst organic ions, the same general conclusions hold for such ions as tetramethyl-ammonium, $\text{N(CH}_3)_4^+$, and acetate, which have relatively low oil-water partition coefficients; these ions have a definite effect on the injury potential which is much more marked in the case of the cations, and which decreases as the size of the cation is increased. The action of these ions with low oil-water partition coefficients is, like that of the inorganic ions, reversible. When the size of a type of organic ion is increased beyond a certain point, the exact point depending on the homologous series chosen, activity increases rapidly, more or less parallel with the increase in oil-water partition coefficient, and at the same time the action of the ion becomes much less reversible.

Concerning the nature of the resting potential a number of different hypotheses have been developed. The first of these, initially due to Ostwald (1890) and Bernstein (1902), is that the membrane acts as a molecular sieve, and that the potential is essentially a diffusion potential. This view has been supported by Höber (1905), Michaelis (1926), Netter (1927) and to some extent by Wilbrandt (1937). An alternative view (Beutner, 1920) is that the membrane is a homogeneous non-aqueous layer, and the potential is a phase-boundary potential; whilst Osterhout (1933) agrees with Beutner that the membrane is non-aqueous, but considers that the potential is a diffusion potential. Thanks to the recent analysis of Teorell and Meyer, we now know that whether the membrane is porous or homogeneous and lipoidal, the potential will consist of two potentials due to uneven distribution of ions across the membrane surface, with a diffusion potential operating in the interior of the membrane. If the membrane is porous, the selective permeability will be due to the pores being of such a size as to be permeable to the small ion K^+ ,

but not to the larger ions Na^+ , Ca^{++} , etc. If it is true that the membrane is relatively impermeable to small anions, the pores must also carry a negative charge to reduce the ease of penetration of anions such as Cl^- which are of about the same size as K^+ . According to the theory of Teorell and Meyer, under these conditions the potential should not obey a linear relationship when plotted against $\log [\text{K}^+]$. Since, however, the potential does appear to obey this relationship, either the theory of Teorell and Meyer cannot be applied to this system, the permeability to anions is greater than is generally supposed, or else the membrane is not porous. If, on the other hand, the membrane is homogeneous, then according to Meyer the specific action of K^+ must be due to selective solubility and high mobility of K^+ in the membrane, and the potential will be proportional to $\log [\text{K}^+]$. The ions, such as Cs^+ and NH_2CH_2^- , which have a low oil-water partition coefficient but which to some extent resemble K^+ , will then have some effect on the potential due to their being to some extent selectively dissolved; the ions such as $(\text{C}_3\text{H}_7)_2\text{NH}_2^+$, which are very different from K^+ in properties, but have still a low oil-water partition coefficient, will have no selective solubility and therefore little action on the potential; finally, ions such as $(\text{C}_5\text{H}_9)_2\text{NH}_2^+$, which have an appreciable oil-water partition coefficient, will again be soluble in the membrane and will affect the resting potential.

The effects due to the anions with rather high oil-water partition coefficients, such as laurate and oleate, are irreversible, and probably reduce the potential observed by cytolysis, i.e. a breaking-up or loosening of the structure of the membrane (Höber, 1937).

We may then distinguish at least three ways by which an ion may affect the potential across a membrane: (1) by virtue of its ability to diffuse, it may affect the membrane potential directly; (2) by interaction with the polar groups and ions of the membrane, it may change the membrane structure and affect the potential indirectly; (3) by dissolving in the substance of the membrane, it may change the membrane structure and affect the potential indirectly.

In view of the relatively unknown character of the nerve and muscle plasma membranes, we do not think that the study of any particular case has yet been sufficiently detailed to permit of distinguishing which of the numerous variables is concerned in

any individual case. Consequently, the only legitimate conclusion which may be drawn from potential measurements is that the membrane is selectively permeable to certain cations, of which K^+ is the outstandingly important member. With regard to anions, the evidence is too limited to warrant any conclusions.

During the transmission of an impulse the resting potential falls transitorily, and then as the impulse passes is restored to its initial value, sometimes after several minor fluctuations known as "after-potentials". Lillie (1923) suggested a theory of transmission of impulses which postulated that this decline in the resting potential was due to a transitory change in membrane permeability. As we have seen earlier in this chapter, such changes in permeability to ions have been observed experimentally by impedance studies. Cowan has shown that K^+ leaks from crab nerve when stimulated, and Fenn (1937) found a leakage of K^+ from stimulated frog muscle, which may be due to the increased permeability to ions revealed by the impedance studies. The identity in time of the fall in membrane impedance and of potassium leakage has not, however, been established. Hill (1932) has measured the heat liberated by the passage of an impulse, finding that, when the heat due to the chemical processes of recovery is excluded, the heat per impulse lies between 5×10^{-3} and 2.5×10^{-4} ergs per sq. cm. of nerve surface. Hill points out that this is only about 1/4000 of the energy of an olive-oil surface. Or, if we take the free surface energy of a typical cell membrane as 0.1 dyne per cm. (Harvey & Danielli, 1938), the energy per impulse is only about 1/50 of the free surface energy of the membrane; i.e. if the area of the surface were decreased by 2%, this amount of heat would be evolved. The heat evolved by disruption of the membrane would be at least twenty-five times larger than this. Consequently, as Hill points out, these figures seem to preclude a complete breakdown of the cell membrane. The same conclusion may be reached from impedance studies, for although the nerve membrane resistance (of a squid nerve) is reduced to about 1% of its resting value during the passage of an impulse, the minimum specific resistance of the membrane is still of the order of 10^9 ohms, compared with 10^2 ohms for $N/10$ KCl.

Large Plant Cells. There are a number of large plant cells, e.g. *Nitella*, *Valonia* and *Halicystis*, that consist of a cellulose wall inside which is a thin layer of protoplasm about 10μ in thickness,

IMPEDANCE AND POTENTIAL MEASUREMENTS

a central vacuole filled with sap. *Valonia* and *Halicystis* are more or less globular, and reach a diameter of several centimetres. *Nitella* forms relatively slender rods, which may, however, be several inches in length. These cells have been extensively investigated by Osterhout and his colleagues (1933, 1937). Typical methods of study are to place electrodes at the two ends of a *Nitella* cell and kill the protoplasm at one end, thus establishing contact with the sap; or to impale a *Valonia* cell on a capillary, through which electrical contact is made with the cell sap. In the case of *Halicystis* two capillaries may be inserted and the sap replaced by other solutions. When the same solution is present on both sides of *Halicystis* protoplasm, a negative asymmetry potential is found of about 70 millivolts when sap is present on both sides. It is changed to a potential of 30 millivolts of the opposite sign when sea water of pH 8.1 is present on both sides of the membrane. Sap is very similar to sea water, except that its pH is about 5-6. With *Nitella*, the asymmetry potential with sap both sides is 15 millivolts negative, and in *Valonia* 65 millivolts positive. Blinks has shown that these potentials fall off in the absence of oxygen. If contact is made at two points on the surface of a *Nitella* cell, as with nerve the potential difference between the two points is very sensitive to the K^+ concentration but not to the Na^+ concentration. Thus, if the potential is first measured with $N\ 100\ KCl$ at one point, and then the KCl concentration is lowered to $N\ 1000$, the potential changes by 55 millivolts. If we substitute this value in the equation

$$E = 0.058 \frac{U_K - U_A}{U_K + U_A} \log \frac{C_1}{C_2},$$

putting the apparent mobility of Cl^- (U_A) = 1, we find the apparent mobility of K^+ $U_K = 85.5$. In the case of $NaCl$ solutions we find the apparent relative mobility of Na^+ to be 2. For *Valonia* the ratios are $K^+ = 20$, $Na^+ = 0.2$, $Cl^- = 1.0$. Thus the protoplasmic surface of these plant cells responds in the same selective manner to K^+ as do the excitable cells of animals. From these apparent mobilities the potentials obtained with mixtures of KCl and $NaCl$ can be calculated and agree to within 5-10% with the observed values. The selective reaction of the cell membrane to K^+ may readily be modified. Osterhout (1936) found that in the presence of $N\ 100$ guaiacol, the mobility of K^+ in the membrane falls and

that of Na^+ rises until the latter becomes the larger. In the case of *Nitella*, which usually grows in pond water, containing some calcium, washing with distilled water or NaCl solution removes a substance which appears to be responsible for the abnormal apparent mobility of K^+ , since, as a result of washing, the mobility of K^+ falls to the same value as that of Na^+ , and the excitability is also lost. Either or both of the excitability and high mobility of K^+ may be restored by adding the substance which was washed out, or by NH_4Cl , $\text{N}^+\text{Et}_4\text{Cl}$, guanidine, adrenalin and ephedrine. Substances present in blood, urine and saliva will also restore the irritability, and Osterhout suggests that these substances may be responsible for the irritability of muscle and nerve.

Meyer & Sievers (1936) have attempted to apply their theory to these large plant cells. With *Nitella*, Osterhout & Harris 1929 obtained the results shown in Table LI. The potential difference in the case of the protoplasmic membrane is very dependent on the absolute concentration, and so it is suggested that the protoplasmic membrane must contain high molecular weight acids which provide fixed anions. With the cell membrane, on the other hand, the effect of concentration is much less and the potential difference may perhaps be due to the sieve effect.

TABLE LI. THE EFFECT OF CONCENTRATION ON THE POTENTIAL-DEVELOPED BY *NITELLA* MEMBRANE

	Concentrations of KCl	Millivolts
Cell membrane	0.1 — 0.001	-1.6
	0.01 — 0.001	-1.65
Protoplasmic membrane	0.1 — 0.01	
	0.01 — 0.001	-56.4

On the other hand, the potential developed across *Valonia* protoplasm when different concentrations of sea water are placed on the two sides is practically independent of concentration (Table LII). The E.M.F. may be calculated from the equation

$$E = \frac{2RT}{zF} \log \frac{C_1}{C_2}$$

so that $A=0$, and the potential (according to Meyer & Sievers) is exclusively a sieve effect and is due to the larger cation Na^+ being held back by the pore size more than is the smaller anion Cl^- .

However, with these plant cells, as with nerve and muscle, the only conclusion drawn from potential studies which appears to be beyond dispute is that the membrane is normally slightly permeable to anions and to cations, that normally the membrane is permeable to K^+ to a degree which implies some specificity in the cell membrane, and that in some cases the addition or subtraction of substances to the cell membrane will reduce its permeability to K^+ to roughly the same value as to Na^+ .

TABLE LIII THE CHANGES IN POTENTIAL ACROSS A MEMBRANE OF THE FROG SKIN WHEN DIFFERENT CONCENTRATIONS OF SEA WATER ENTER THE TWO SIDES

Concentrations of sea water		Millivolts
1.5	0.5	17.3
1.0	0.33	19
0.67	0.23	19

Frog Skin Potentials. If the same Ringer's solution is placed on both sides of a frog-skin membrane, a potential of some millivolts is found between the two sides. This potential has been the subject of many investigations, but it cannot yet be said that the results give any particular clue as to the permeability of the membrane. This is hardly surprising for, as we have seen, even in the case of single cells such as the large plant cells, or in the case of arrays of similar cells, such as is found in a nerve trunk, the interpretation of the potentials observed is still in its infancy. Frog skin consists of a large number of different types of cell—smooth muscle cells, contractile melanophores, cells whose chief function is to provide an inert outer surface to the skin, other cells whose chief function is secretion of various products, capillary endothelial cells, etc.—and these cells are arranged sometimes in parallel, sometimes in series. The membranes of these different types of cell are probably all different, and probably respond in different ways to the same change in their environment. In presenting the following data, therefore, we do so not with any hope of an immediate interpretation, but rather to exhibit the complexity of the experimental results.

Dead frog skin has been examined by Amberson & Klein (1928) and Motokawa (1933) and found to behave like an amphoteric membrane, preferentially permeable to cations when dissociated

as an acid in more alkaline solutions, and to anions when dissociated as a base in more acid solutions.

The behaviour of living frog skin is in most respects quite different from that of the dead skin. It is not greatly affected by variation of pH between 7.6 and 8.6. When Ringer is present on both sides of the skin at pH 8, the potential across the skin is about $+40$ millivolts (the inner side of the skin being considered as positive). Change of pH outside the previously mentioned limits, or replacement of the Na^+ of the Ringer by Ca^{++} , Mg^{++} , NH_4^+ , Rb^+ or Cs^+ , causes a fairly reproducible fall of potential to a value a few millivolts positive, whilst K^+ reduces the potential to zero. The latter observation is most important, since we have seen for simpler systems that this is a characteristic effect of K^+ on cell membranes; furthermore, as in the case of cell membranes, K^+ greatly reduces the electrical resistance. The potential is also dependent on respiration. These three facts alone are probably sufficient to locate the site of origin of the potential at the membranes of the various cells present. Since the membranes of the different cells are probably different in their electrochemical behaviour, giving the total membrane a mosaic character, quantitative theoretical treatments, such as have been used by Osterhout for plant cells, cannot be applied to frog skin.

When Ringer is present at the inner surface and greatly diluted Ringer at the outer surface, the potential is lower than that with Ringer at both surfaces. According to Dean & Gatty (1927) it may reach values of -30 or -40 millivolts when Na^+ is absent from the dilute side. When a sodium-free solution is replaced by a sodium-rich solution, the potential rises fairly rapidly to a maximum, more positive value, and then falls off more slowly to a final potential which lies between the initial potential and the maximum potential. Dean & Gatty, and Hashida (1922), give results for a number of other cations and anions, which in some cases conflict.

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THE EFFECTS OF NARCOTIC SUBSTANCES

Alcock (1906) observed a reversible decrease in the resting potential of frog nerve on treatment with chloroform and ether vapours; these and other results he interpreted as being due to an increased permeability of the tissue to ions. Galeotti & Di Cristina (1910) observed that local narcotisation of muscle causes the spot so treated to become negative with regard to the rest of the muscle; this effect was never completely reversible and it would seem that the effect is similar to the irreversible damaging of muscle by cutting it, etc. Höber (1907), on the basis of the study of "salt effects" concluded that narcotics decrease permeability and he attempted to relate the activity of narcotics with their activity on colloidal systems.

Lillie, in a series of papers (see his monograph, 1923), developed the theory which has come to be known as the "Permeability Theory of Narcosis" on the basis of measurements of changes in apparent permeability and physiological behaviour. Lillie's indirect studies were on *Arenicola* larvae, and he observed a strict parallelism between the action of narcotics in preventing contraction of the body wall on transference of the worm to pure sodium-chloride solution, and in preventing the escape of pigment from the larvae, which occurs at the same time. Similarly, studies on the formation of artificial fertilisation membranes in *Asterias* eggs led Lillie to believe in the close connection between permeability changes and narcosis. Lillie's view was that a narcotic tends to prevent a rise in permeability which occurs during activity, and it does this in virtue of its accumulation in the lipid phase of the membrane. Thus a narcotic will, according to this view, decrease the irritability of a tissue, since irritability is associated with the power of the cell membrane to become more permeable on stimulation, and furthermore, it will tend to have an anti-toxic action against substances which normally cause cytolysis.

Passing now to more direct studies of the effect of narcotics on permeability, we may first consider the permeability of various cells to water. Winterstein (1916) measured the rate of increase of weight of frog sartorius muscles in hypotonic salt solutions and concluded that the rate of increase, i.e. the permeability to water, was smaller when alcohol was added. For example, a muscle in 0.35% sodium chloride showed an increase in weight of 32.3% in 1 hour, whereas a similar muscle in 0.35% sodium chloride

+4% ethyl alcohol increased in weight by only 22% in the same time. Winterstein also constructed artificial cells by covering the ends of glass tubes with abdominal muscles of frogs: by placing a salt solution inside the "cell" so formed and placing the latter in hypotonic solution, the migration of water across the muscle could be measured by weighing the cell. Migration of salts was measured by analysis. In Table LIII a typical experiment is shown, and it may be seen that the 6% ethyl alcohol exerted a reversible inhibitory action on the permeability to water.

TABLE LIII. THE EFFECT OF ALCOHOL ON THE DIFFUSION OF WATER THROUGH THE ABDOMINAL MUSCLES OF FROGS. WINTERSTEIN, 1919

	Experimental experiment "Cells" filled with 0.7% NaCl + 6 vol. % alcohol placed for 1 hour in distilled water containing 6 vol. % alcohol	Control experiment: "Cells" filled with 0.7% NaCl and placed for 1 hour in distilled water
	6%	6%
Water intake	1.4	7.2
Diffusion of salt	1.1	1.6
Final concentration of NaCl	0.68	0.64

At the end of this experiment the muscle membranes of the "cells" were placed for 50 min. in 0.7% NaCl. Both cells were then filled with 0.7% NaCl and placed in distilled water for 1 hour.

Water intake	8.1	8.3
Diffusion of salt	0.1	4
Final concentration of NaCl	0.65	0.4

In Table LIV are shown some of Lillie's (1918) results on the permeability of *Arbacia* eggs to water. Lillie found that whereas narcotics do not decrease the permeability of these eggs to water when placed in diluted sea water, these substances do prevent the increase in permeability caused by fertilisation of the eggs. The table compares the concentrations of various narcotics required to prevent the increase in permeability on fertilisation with the concentrations required to prevent cleavage of the eggs, and a strong parallelism is evident. Lucké (1931) also found that in diluted sea water narcotic substances only caused an irreversible increase in permeability to water; however, if the permeability to water is increased by placing the eggs in a non-electrolyte medium (Chapter x), it was found that substituted urethanes (carbamates) caused a reversible decrease in permeability. The work of Lillie and of Lucké demonstrates a very interesting point

in that it shows that in its normal environment the *Arbacia* egg's permeability to water is uninfluenced by narcotic substances except for irreversible changes when toxic concentrations are used, but that when the permeability is increased, either by replacing the sea water with a non-electrolyte medium or by fertilisation, the narcotics are then effective in retarding the rate of penetration of water.

TABLE LIV. COMPARISON OF THE CONCENTRATIONS OF CERTAIN ANAESTHETICS REQUIRED TO PREVENT THE INCREASE IN PERMEABILITY OF *Arbacia* EGGS TO WATER FOLLOWING FERTILISATION WITH THE CONCENTRATIONS NECESSARY TO INHIBIT CELL CLEAVAGE (LILLIE, 1948)

Anaesthetic	Concentration to prevent increase in permeability due to fertilisation	Concentration to prevent cleavage of fertilised eggs
Chloral hydrate	ca. 0.2%	0.1-0.2%
Chloroform	1:10 sat. 0.05%	0.05%
Methyl alcohol	8%	.
Ethyl alcohol	5%	5%
n-Propyl alcohol	2%	2%
i-Butyl alcohol	1-1.2%	.
n-Amyl alcohol	0.6%	ca. 0.4%
Ethyl carbamate	2%	1.5-1.75%
Ether	1.2-1.4%	0.5-0.6%

The influence of narcotics on the permeability of the ox erythrocyte to water has been studied in a satisfactory manner by Jacobs & Parpart (1932), and they have shown that the inhibition of osmotic haemolysis by narcotics, observed by many workers previously, is due mainly to a change in the critical haemolytic volume of the cells rather than to a decrease in the permeability to water. This point is made clear by Fig. 55, in which the percentage haemolysis of cells in 0.09 *M* sodium chloride with and without 0.3 *M* ethyl carbamate is plotted against time. The curves show that the cells to which the carbamate had been added haemolysed to a less extent than the untreated ones; the comparative rates at which haemolysis occurred initially are apparently not markedly different, so that the influence of the carbamate on water permeability is very small, if there is any influence at all.

With regard to the permeability of cells to non-electrolytes, it appears that inhibition by narcotics is not so general a phenomenon as was originally thought. Thus Katz (1918) was unable to demonstrate any effect of heptyl alcohol or of thymol on the

penetration of glucose into human cells. Lullies (1925) states that *iso*-butyl carbamate (0.05 to $0.025 M$), phenyl urea ($0.025 M$), heptyl alcohol ($0.017 M$) and propyl alcohol ($0.41 M$) decrease the rate of penetration of glycerol and ethylene glycol into the leaf cells of *Tradescantia discolor*. The narcotics had no measurable effect on urea penetration. Lullies used as his criterion of penetration the de-plasmolysis technique of Fitting (1915), and his results are not above criticism, since the concentrations of the narcotic which were effective were very close to those which produce toxic effects; it is quite possible that the narcotics caused

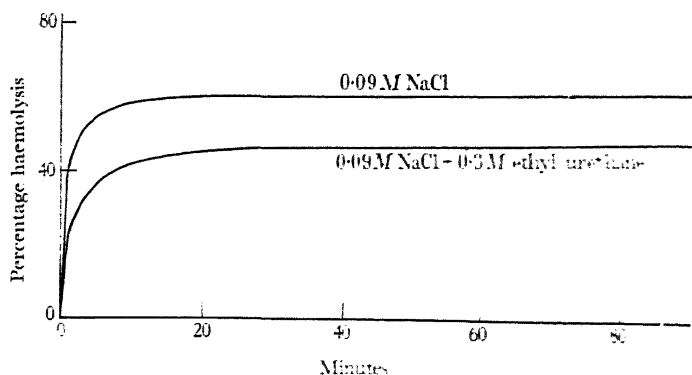


FIG. 55. The effect of ethyl carbamate on plasmolysed onion cells (after Jacobs & Pargant, 1932).

an escape of salts from the plant cells, thereby delaying the de-plasmolysis. Lepeschkin (1911) found that the permeability of *Spirogyra* cells to methylene blue and methyl green was diminished by narcotics, whereas with the lipoid soluble dye, Bismarck brown, no effect was observed; this author thought that narcotic action, so far as permeability diminution was concerned, was only found with lipoid insoluble substances. These results were not confirmed by the investigation of Ruhland (1912), whilst Collander (1921) brought forward evidence which tended to confirm them.

Anselmino (1928) has shown that the permeability of collodion membranes to glucose is decreased by $0.1 M$ amyl alcohol, $0.04 M$ phenyl urethane, and $0.1 M$ *iso*-butyl carbamate; in general the concentrations at which different narcotics were effective were

such as to give the same lowering of the water-air interfacial tension. This author viewed the process of narcosis as a blocking of pores by the narcotic. Anselmino & Hoenig (1936) extended these investigations on artificial membranes to the erythrocyte. The permeability of this cell to glycerol was measured by determining the rate of haemolysis in 2.3 molar solution (the haemolysis technique); the penetration of erythritol, arabinose, xylose, glucose and mannose by the change in volume as measured by the haematocrite. The retarding effect of varying concentrations of ethyl, propyl and butyl carbamates on the time of haemolysis of human cells in pure glycerol solution are quite striking. The effects of the other substances studied were very much smaller, but reproducible. The homologous alcohols behaved in the same way as the urethanes. Jacobs & Parpart (1937) have extended the investigations of Anselmino & Hoenig to a large number of species and to the study of different substances. In a preliminary report of their findings, Jacobs & Parpart state: "The present study, involving the erythrocytes of a number of species of mammals and of some other vertebrates, shows somewhat more complicated conditions than those brought out by Anselmino & Hoenig. Thus while *n*-butyl alcohol in concentrations from 0.0156 *M* to 0.25 *M* may greatly decrease the permeability of the erythrocytes not only of man but also of the rat, rabbit, guinea-pig, ground-hog, and several birds, the opposite effect is obtained with the erythrocytes of the ox, sheep, pig, horse, dog, cat, and several reptiles and fishes. In general these two groups of species are the same as those already distinguished by other properties of their erythrocytes (see Chapter VIII). In several cases involving decreased permeability, the order of effectiveness of a series of alcohols is: methyl < ethyl < propyl < butyl < amyl. With erythrocytes of the ground-hog, the effectiveness of *n*-butyl alcohol increases with increasing molecular weight of the penetrating substance in the order: ethylene glycol < glycerol < erythritol < mannitol. As contrasted with glycerol and related substances to which *n*-butyl alcohol increases the permeability in some species and decreases it in others, thiourea, under the same conditions, always shows an increased permeability. The same was found to be true of lipid soluble substances such as mono-acetin and ammonium salts of weak acids, which by hydrolysis give rise to, and lipid soluble acids. On the contrary, permeability to

the ammonium salts of strong acids, where the penetration of the cell by ions is presumably involved, was in all species found to be greatly decreased." Where butyl alcohol slightly increases permeability to lipid soluble substances, it usually greatly decreases permeability to anions.

Some results of Bärhund (1938) on *Chara ceratophylla* are of particular interest, since from an experimental point of view they are the most satisfactory in the literature on the influence of narcotics on non-electrolyte permeability, because they were carried out on the cell in its normal environment and the penetrating substances were determined chemically. Some typical results are shown in Table LV, and it is clear that the only influence of ether, the narcotic substance used throughout this work, is to increase permeability, whether the relatively lipid insoluble ethylene glycol or the relatively lipid soluble triethyl citrate is considered. Bärhund observed that the acceleration in permeability is reversible, and he was able to correlate the effects on permeability with the inhibiting action of the narcotic on protoplasmic streaming.

TABLE LV. THE EFFECT OF ETHER ON THE PERMEABILITY OF (*C. CERATOPHYLLA*) TO NON-ELECTROLYTES

P_1 is the permeability constant in the absence of ether. r is the ratio P_2/P_1 .

Penetrating substance	Ether concn. vol.-%	P_1	P_2	r
Ethylene glycol	0.5	1.14	0.87	0.76
	1.0	1.27	0.87	0.68
	1.5	1.27	0.61	0.48
	2.0	3.28	1.28	0.39
	2.5	3.41	0.98	0.29
Triethyl citrate	2.5	9.27	6.16	0.66
	2.5	10.35	5.46	0.53
	2.5	9.85	8.25	0.84
Hexamethylene tetramine	2.5	0.07	0.0270	0.39
	2.5	0.241	0.0495	0.21
Urea	2.5	0.179	0.163	0.91
	2.5	0.104	0.0678	0.65

The influence of narcotics on the penetration of CO_2 has been demonstrated by Smith (1923); this author made use of the colour change which occurs inside the cells of the petals of *Ipomoea Learii* when they become acid as a result of the penetration of this gas. It was found that placing the cells in CO_2 -saturated water in

which 0.04-0.1 M CHCl_3 or ether had been added considerably delayed the appearance of the colour change; generally in low concentrations of the narcotic the effect was reversible; in higher it was irreversible.

Passing now to the permeability of cells to substances which dissociate in solution, we have the observation of Lepeschkin (1911) that the permeability of *Tradescantia* cells to NaNO_3 is decreased by 1-2.5% ether and 0.05-0.12% chloroform; if the chloroform concentration was increased to 0.2%, an increase in permeability was measured. The conductivity studies of Osterhout have already been mentioned in the chapter on ionic permeability; this author (1913) found a transient increase in the electrical resistance of *Lemna* fronds with various narcotics; this change was, however, followed by an irreversible increase in conductivity and it is difficult to state with certainty whether the results can be related to ionic permeability changes. Joel (1915) washed ox erythrocytes repeatedly with non-electrolyte solution and then measured the electrical conductivity of a suspension of the washed cells in a fresh lot of non-electrolyte solution. We have already seen that suspension of unwashed cells in a non-electrolyte medium causes an escape of salts from the ox erythrocyte; Joel's treatment was therefore quite drastic, and it is difficult to be sure that the increase in conductivity observed was due to a permeability to salts in the true sense, in contrast with actual haemolysis. Joel observed that additions of urethanes, thymol and alcohols all tended to decrease the rate of rise of conductivity, indicating either an inhibition of the permeability of the cells to salts or an inhibition of haemolysis.

Siebeck (1922) has studied by direct chemical methods the effect of narcotics on the rate of exchange of sulphate with chloride across the erythrocyte membrane, and he found a definite reversible decrease in the rate when the urethanes, substituted ureas and alcohols were added to the medium; the concentrations which were effective were identical with those required to inhibit cell oxidation.

Tröndle (1920) has studied the effect of ether and chloral hydrate on the penetration of NaCl , KCl and NaNO_3 into the palisade cells of *Buxus sempervirens*; the method consisted in measuring the limiting plasmolytic concentration, i.e. the smallest concentration of a salt which is necessary to produce plasmolysis,

at intervals during the soaking of the leaves in the solutions of the penetrating substances. Tröndle found that the uptake of salts, measured in this way, could be almost completely inhibited by previous treatment of the cells with 1% chloral hydrate or 3% ether. Similar results were found with leaves of *Acer platanoides*, and the roots of *Lupinus albus*. The penetration of alkaloids, on the other hand, was found to be uninfluenced by chloral hydrate, and Tröndle argued that since alkaloids penetrate without the intervention of any metabolic process, in contrast with salts whose penetration is determined, so he claimed, by metabolic activity, narcotic action would only be manifested in regard to the penetration of substances whose penetration involved such a metabolic activity. A similar view in regard to animal cells has been propounded by Höber (1922). We have had occasion to mention earlier the results of Davson (1940) on the relative influences of amyl alcohol on the permeability of the cat erythrocyte to sodium and potassium, and it was shown that narcotics only cause an increase in potassium permeability whilst, in the same concentrations as this increase is observed, an inhibition of sodium permeability occurs. In Fig. 56 is shown the effects of three homologous carbamates on sodium permeability P_{Na} ; in this figure P_{Na} is plotted against the concentration of the carbamates in the isotonic KCl suspension medium, and the units of concentration of these substances are chosen in such a way that at any point on the curve they bear the relationship:

$$C_{Et} : C_{Pr} : C_{Bu} = 1 : 1.25 : 1.25.$$

and the close approximation of the points to each other indicates that Traube's Rule is obeyed. A large variety of organic substances had a similar inhibiting action on sodium permeability, e.g. chloroform, ether, benzene, alcohols, polyphenols, etc. The inhibiting action of anions, e.g. CNS^- and I^- , mentioned in Chapter XII may probably be classed as a narcotic action in the sense in which the word is used in this chapter, and it is worthy of note that the adsorbable anions phenylacetate, iodoacetate, and mono-chloracetate exert an inhibiting action; similarly the surface-active soaps such as sodium oleate have an inhibiting action.

An interesting point arising out of this work is that saponin is completely without effect either on sodium or potassium permeability; on the other hand, a soap, such as sodium oleate,

behaves in a similar way to the alcohols and carbamates in it inhibits sodium permeability and only accelerates potassium

In earlier chapters the apparently unique behaviour of saponin in respect to the pre-haemolytic escape of potassium from the rabbit erythrocyte, and the penetration of lipoid protein films, has been indicated; thus, although a variety of haemolytic agents will cause the rabbit erythrocyte to lose potassium before

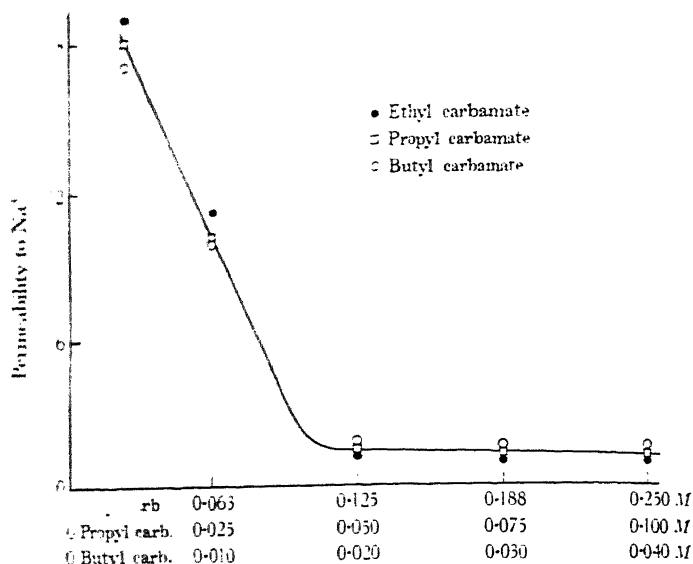


FIG. 56. Effect of carbamates on permeability of cat red cells to sodium.

haemolysis occurs, saponin does not have this effect; again, a variety of haemolytic substances will penetrate a lipoid-protein film, whilst saponin does not. Unpublished results of Davson also show that the failure of saponin to inhibit penetration is not confined to this particular system, but extends to the penetration of non-electrolytes such as glycerol into the rabbit erythrocyte. A further class of substances which has been found to inhibit the penetration of sodium from the cat erythrocyte is represented by the heavy metals; thus copper, lead and zinc in concentrations from 1×10^{-4} to $1 \times 10^{-6} M$ all cause a marked slowing of the rate of escape of sodium. Potassium permeability, as with all the other

substances mentioned, being markedly increased. Mention has already been made of a similar action of copper on the permeability of the rabbit erythrocyte to glycerol, and it would seem from the studies of Jacobs that inhibition of glycerol permeability by copper runs parallel with inhibition by narcotic substances, i.e. the cells of those species, e.g. the rabbit, human, rat, etc., whose permeability to glycerol is inhibited by copper also show the phenomenon of inhibition of glycerol permeability by narcotics. It is worthy of note that the cat erythrocyte's permeability to glycerol is only increased by narcotics or copper, so that a given species of erythrocyte may show a narcotic effect with regard to certain solutes and not with regard to others.

So far as the interpretation of the action of narcotic substances on permeability is concerned, it may be stated without much hesitation that no single theory propounded in the literature covers all the facts. Speculation has been particularly prevalent in this field of permeability, and practically every paper to which reference has been made contains a lengthy discussion of the particular results described with special reference to the particular theory favoured by the author. The summary of the facts presented here will enable us to see the inadequacy of some of the theories. The idea developed by Höber and by Tröndle, that narcotic action on permeability was confined to those substances whose penetration is determined by metabolic processes, is incorrect—since Anselmino & Hoening and Jacobs & Purpura have shown that the penetration of glycerol into the erythrocyte may be inhibited by narcotic substances. The pore blocking theory of Anselmino is also incorrect in so far as biological systems are concerned, since Davson has shown that a narcotic may inhibit the penetration of sodium whilst it accelerates the penetration of potassium.

The particular physical characteristics of a substance which determine whether it will be a narcotic substance have also been the subject of much discussion. Traube suggested that the surface activity was important, and the evidence in the literature certainly indicates that this is a factor of importance; in general, in a homologous series, the effective concentrations of the different members are approximately iso-capillary; however, the fact that ether and chloroform, which do not concentrate at an oil-water interface, are effective narcotic substances indicates that surface

activity is not the sole factor: further saponin is strongly surface-active but has no narcotic action on permeability. In a similar way, the new law that regards the determining factor as the lipoid solubility of the substance is in accord with most of the facts in systems which show the phenomenon of the inhibition of permeability by narcotic substances, although saponin is, once again, an exception. The fact that certain cells show no evidence of an inhibition of permeability by the narcotic substances examined, and that other cells only show this inhibition in respect to certain penetrating substances, indicates that besides the chemical nature of the narcotic substance, the special structure of the membrane and that of the penetrating substance must also be taken into account. At this point it is of interest to re-examine Lillie's hypothesis regarding narcotic action and permeability. It will be remembered that Lillie supposed that the action of a narcotic substance was a preventive one, in that it inhibited an increase of permeability which would occur in the absence of the narcotic. Thus a narcotic would prevent the conduction of an impulse through a nerve, not because it altered the normal permeability relations in the resting nerve, but because it prevented the supposed increase in permeability which is associated with the passage of the impulse. If Lillie's view is correct, it would be expected that the cell in its normal environment will show no effect of narcotic substances on its permeability. In support of this idea we find that the one study of non-electrolyte permeability which has been carried out under strictly normal conditions, namely that of Bärhund, indicates that narcotics do not decrease permeability. The studies of Anselmino & Hoenig and of Jacobs & Parpart were carried out in electrolyte-free solutions, and since cells are not normal in these circumstances it may be that the narcotic inhibits an increase in permeability due to these conditions. Similarly, the work of Davson on narcotic action was carried out in potassium-chloride solutions, and the work of this author indicates that whereas the penetration of potassium is not affected by potassium concentration (apart from the effects expected on the basis of altered concentration differences), that of sodium is, and it is interesting that potassium permeability is not retarded by narcotics whilst sodium permeability is, and in a very striking manner. Again Lillie's and Lucké's work on unfertilised eggs of *Arbacia* has shown that the permeability of

these eggs to water is unaffected by narcotics when the egg is in its normal environment; only when the permeability is increased by fertilisation or by placing the cells in a non-electrolyte medium does the narcotic exert an inhibiting action on water permeability. These facts are certainly suggestive, but the fact that collodion membranes show narcotic inhibition of permeability, and the well-established inhibition of the chloride-sulphate exchange in the erythrocyte, indicate that the application of Lillie's idea is limited.

In concluding, we may state that the experimental work on the influence of narcotic substances on permeability is still too limited to warrant any generalisations either regarding the mechanism of this action or regarding the significance of these changes in respect to narcosis itself; the use of the exact methods, such as those developed by Collander and Bärlund, by Lucké and by Jacobs, applied with care to different cells under strictly physiological conditions should lead to a rapid advance in this branch of permeability.

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CHAPTER XVII

THE EFFECT OF TEMPERATURE ON PERMEABILITY

By J. F. DANIELLI and H. DAVSON

A RISE in temperature increases the kinetic energy of molecules and ions present in a solution. The kinetic energy is, of course, expressible in terms of mass and velocity. In a perfect gas the kinetic energy per molecule is given by

$$\text{K.E.} = \frac{1}{2}nm\bar{u}^2,$$

where n is the number of molecules in a given volume, m is the mass of the molecule and \bar{u} is the root mean square velocity.

The relationship between \bar{u} and temperature is given by expressing Boyle's and Charles' Laws in terms of molecular kinetics:

$$\frac{1}{2}Nn\bar{u}^2 = RT, \text{ where } N = \text{Avogadro's number.}$$

Consequently, for two different temperatures, T^+ and T^- , we have the following, since R is a constant:

$$\bar{u}_T \propto \sqrt{T}$$

The ratio \bar{u}_{T-10}/\bar{u}_T is known as the temperature coefficient and is symbolised by Q_{10} . Between 20° and 30° it should have a value of 1.02. This simple treatment assumes that there is no increase in the vibrational energy of the molecules with the rise in temperature. If the laws for perfect gases applied for dilute solutions, then for a simple diffusion process the Q_{10} should be of this order.

In actual fact, as we saw in Chapter v, the Q_{10} of diffusion in water at 20° C. lies between 1.2 for rapidly diffusing molecules and 1.5 for very slow molecules. This deviation from the value of 1.02 found for perfect gases is due to the restraint placed on free diffusion by the structure of the liquid. Very much higher coefficients may be obtained with media in which the structure is more rigid, so that a higher kinetic energy per molecule is required before a diffusing molecule can break away from the local structure. Some such results of Barrer (1934) are given for

silica glass in Table LVI. As we have pointed out in Chapters v and vii, the temperature coefficient is a most valuable quantity, since it enables one to estimate the minimum energy required by a molecule before it is free to diffuse, and this is necessary for the quantitative examination of membrane structure. For example, with silica glass the values of $PM^{\frac{1}{2}}Q_{40}^{T-40,40}$ are roughly the same for members of the group H_2 and helium, and for members of the group nitrogen, oxygen, argon, but different for the two groups. We conclude, therefore, that the membrane is unhomogeneous, and that probably H_2 and helium diffuse mainly by one path or mechanism, and that N_2 , O_2 and argon diffuse mainly by a different path or mechanism. Barrer had previously concluded on other grounds that the first group of molecules penetrates through the crystal lattice, and that the other group can only penetrate through the interspaces between the crystallites composing the membrane.

TABLE LVI. DATA FOR DIFFUSION THROUGH SILICA GLASS MEMBRANES AT 500° C.

Substance	$P \times 10^4$	Q_{40}	
Helium	1.55	1.55	1
Hydrogen	0.5	2.19	10^2
Nitrogen	0.001		
Oxygen			
Argon			

TABLE LVII. THE EFFECT OF TEMPERATURE ON THE PERMEABILITY OF THE ERYTHROCYTE TO GLUCOSE

Temperature	Rate of penetration of glucose		
0.5°	1	12	$0.5^{\circ}-15^{\circ}$
5.5°	4		
10.0°	12	6	$5.5^{\circ}-15^{\circ}$
15.0°	24	2.7	$10^{\circ}-25^{\circ}$
25.0°	54	2.25	$15^{\circ}-25^{\circ}$

Masing (1914) found the results given in Table LVII for the effect of temperature on the penetration of glucose into the human erythrocyte. The Q_{10} values are very approximate owing to the poor experimental conditions. Here it is quite obvious that the Q_{10} is of quite another order from that of simple diffusion in water or gases. For Cl^- a value of 2.2 is given by Ege (1922). This value must be accepted with caution, however, as it was obtained by measurements of the rate of haemolysis in NH_4Cl , a method to

which there are objections. The Q_{10} for the penetration of K^+ through the rabbit erythrocyte membrane in hypotonic solution has been mentioned earlier and has a value of about 2 (Davson, 1937). The membrane under these conditions is probably slightly stretched.

The most systematic study of the effect of temperature on the rate of haemolysis in solutions of penetrating substances has been made by Jacobs *et al.* (1935) and a few of their results have been given in Table LVIII.

TABLE LVIII. Q_{10} VALUES FOR THE TIME OF HAEMOLYSIS OF OX RED CELLS IN WATER AND IN 0.3M SOLUTIONS OF VARIOUS SOLUTES

Temperature interval:	0-10° C.	10-20° C.	20-30° C.	30-40° C.	40-50° C.
Water	1.18	1.30	.	.	.
Propyl alcohol	1.48	1.10	.	.	.
Urea	1.31	1.45	1.38	.	.
Thiourea	2.21	2.64	2.13	1.78	2.10
$\alpha\beta$ Dioxyp propane	3.83	3.07	3.11	2.0	1.83
$\alpha\gamma$ Dioxyp propane	2.92	3.66	3.06	2.59	2.09
Triethylene glycol	3.35	3.62	3.14	2.8	2.24
Diethylene glycol	2.9	2.99	3.12	2.37	2.33
Ethylene glycol	2.81	3.11	2.63	2.37	2.0
Glycerol	5.37	3.98	3.32	2.94	2.74

From this study Jacobs *et al.* conclude that substances which penetrate slowly have a high Q_{10} , and for a given substance and different erythrocytes, the erythrocyte which shows the slower rate of penetration generally has the larger Q_{10} , as was predicted by Danielli (1935). However, the results of Jacobs *et al.* can only be considered approximate, since the haemolysis technique was used, and Davson (1939) has shown that, owing to the escape of cations from erythrocytes in non-electrolyte solutions, the observed effect of temperature on the rate of haemolysis will be complex and will not be due entirely to an increase in the rate of penetration of the non-electrolyte; a close inspection of the results of Jacobs *et al.* shows many deviations from the two generalisations made by Danielli. In fact, with human and rat cells an actual decrease in the rate of haemolysis with a rise of temperature of 10° may be observed. Jacobs *et al.* (1936, 1938) have discussed a number of other factors which affect the observed Q_{10} 's with red cells.

With regard to the effect of temperature, red cells appear to fall into two groups: (1) man, rat, guinea-pig and rabbit; (2) cat,

dog, horse, pig, goat, sheep and ox. The first group differs markedly from the second in the effect, and reversibility of the effect, of temperature. The first group is also highly permeable to glycerol, but this high permeability is sharply reduced by acid or traces of copper. When the permeability of these cells to glycerol has been reduced by one of these agents, the Q_{12} is increased from a low value typical of normal cells of the first group to high values between 3 and 4 typical of the second group of species.

TABLE LIX. VARIATION OF k , THE PERMEABILITY CONSTANT OF *ARBACIA* EGG TO WATER, WITH TEMPERATURE LUCKÉ, HARTLINE & McCUTCHEON, 1931)

	Temperature °C.	k	
Course of swelling	12	0.047	2.1
	15	0.079	2.6
	18	0.079	3.1
	21	0.111	3.1
	24	0.156	
Course of shrinking	12	0.059	1.5
	15	0.067	2.3
	18	0.086	2.8
	21	0.128	3.8
	24	0.157	2.0

TABLE LX. TEMPERATURE COEFFICIENTS FOR THE PENETRATION OF WATER (SHRINKAGE) FROM THE ONION LEAF AND THE SCAPE OF THE DANDELION Delf, 1916

Temperature °C.	Onion %	Dandelion %
5-15	1.4	2.7
10-20	1.5	3.0
15-25	2.0	3.8
20-30	2.6	3.0
25-35	2.9	2.0
30-40	3.0	

In Chapter viii we have given values for the Q_{12} of permeability of *Arbacia* eggs to non-electrolytes. The effect of temperature on the permeability of these eggs to water has been studied in some detail by Lucké *et al.* (1931) and some of their results are given in Table LIX. In plant cells Delf (1916) has measured the effect of temperature on the permeability of onion leaf and dandelion scape and some results are shown in Table LX. Generally speaking there is a great paucity of information about the effect of temperature on permeability constants, and the most systematic investigation is that of Jacobs and his colleagues on red-blood cells.

In the case of the examples of Q_{10} values which have been given here, it has been assumed that the whole effect of temperature change is on the rate of diffusion of the penetrating molecules, and that changes in the cell may be neglected. Probably, however, the structure of the membrane and the equilibrium conditions of the cell are both affected by temperature, and variations in these two quantities will also affect the observed Q_{10} values to some extent. A discussion of some of the theoretical relationships between temperature and permeability is given by Danielli 1941 and Appendix A.

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CHAPTER XVIII

*HAEMOLYSIS**

By H. DAVSON

IN the preceding chapters we have seen the application of two methods whereby the nature of the membrane surrounding a cell may be determined. The first is the study of the permeability of the membrane to a variety of penetrating substances, and the second is the use of electrical, optical and surface tension studies on the membrane itself. There is a third method, which may be spoken of as a degradative method, by analogy with a similar procedure in organic chemistry, which consists in the destruction, either complete or partial, of the membrane under a variety of conditions. It may be expected that an accurate knowledge of the ways in which the membrane may be destroyed, so far as its selective powers are concerned, and the steps whereby this destruction may be brought about, will add to our knowledge of its original structure. Haemolysis describes the change in the erythrocyte which causes the leakage from it of the large molecule of haemoglobin, and this change consists essentially in an actual destruction of the membrane whereby it loses its original selective powers; hence the study of haemolysis is in fact the application of this degradative form of analysis to the problem of membrane structure.

Progress in the elucidation of the mechanism of haemolysis is of only recent date, although the problem has attracted the interest of biological workers since the inception of serious work in this branch of science. The main reason for this delay has been a fundamental misconception as to the nature of the process of haemolysis, which was regarded as a simple permeability process (so that a certain rate of haemolysis was represented as a certain degree of permeability to haemoglobin), whereas more recent evidence indicates that the escape results from the formation of holes in the membrane which are large compared with the haemoglobin molecule so that the escape is probably determined

* We are indebted to Dr Eric Ponder for his criticisms of the first draft of this chapter.

by simple laws of diffusion in an aqueous medium, as opposed to the special case of a permeability process. Again, the majority of workers on haemolysis failed to take into account the effects of temperature and pH changes on the equilibrium conditions of the erythrocyte: thus, if cells are found to haemolyse in 0.08M NaCl at 25° C., it will be observed that they fail to do so on raising the temperature to 40° C.; this effect of temperature used to be attributed to a direct action on the membrane whereby the permeability of the cell to haemoglobin changed, but it may be better accounted for on the basis of a change in the amount of osmotic material in the cell due to a change in the base-binding power of the haemoglobin at the higher temperature. Lastly, no distinction used to be made between the rate at which a haemolytic process occurred, and the equilibrium position achieved, and it is thanks to the pioneering work of Ponder that the process of haemolysis can be characterised by certain curves which contribute largely to the understanding of the changes involved.

The literature on haemolysis is immense, and has been covered in a monograph by Ponder (1934*a*) and a review (1936); in the following pages only that experimental work which appears to be closely related to permeability problems will be dealt with.

We have seen that an erythrocyte can apparently exert no appreciable resistance to an osmotic force, except by allowing water to penetrate it, thereby reducing its internal concentration and hence the osmotic pressure difference between it and its surroundings. We have also seen that there is a limit to the amount of extra water a given cell can hold, and when that limit has been exceeded the membrane apparently gives way and allows haemoglobin to escape. The escape of haemoglobin produced in this way is characterised as hypotonic or osmotic haemolysis. Microscopical investigation shows that the cell leaves a residue behind it after haemolysis: this residue is known as the erythrocyte "ghost", and centrifuging of a haemolysed suspension of cells causes the ghosts to settle to the bottom of the centrifuge tube. Jorpes (1932) has shown that the ghost contains a protein, stromatin, with an iso-electric point of pH 5.5; Boehm (1934) has provided evidence that the protein must exist in a gel state distributed throughout most, if not all, of the interior of the cell—the ghost also contains cholesterol and lecithin, indicating that the

membrane constituents remain attached to the stromatin (Erickson *et al.* 1938). Fricke *et al.* (1939) have studied the ghosts in greater detail and find that the average value of the weight of the "fixed framework" of the erythrocyte is 1.2×10^{-6} grams per square centimetre of cell surface. They point out that if this material were concentrated in the cell surface it would have a thickness of 120 Å; this value, however, excluded any contribution of water.

Spiro (1894) pointed out that if cells were haemolysed in this way, addition of a concentrated NaCl solution to the haemolysed suspension caused a reversal of the process of haemolysis and haemoglobin apparently passed back into the ghosts, which then became impermeable to haemoglobin and were apparently normal erythrocytes again. This idea was revived by Brinkman & Szent-Györgyi (1923), but Bayliss (1924) showed that the addition of NaCl to the haemolysed suspension caused the ghosts to contract in volume and, presumably as a result of this contraction, to become impermeable to haemoglobin. Owing to the loss of water from the cells produced in this way, the concentration of haemoglobin in the ghosts would be higher than that in the suspension medium. There was thus no question of the haemoglobin passing back into the ghosts, but rather a shrinking of the latter accompanied by a resumption of the cell's impermeability to haemoglobin, the two effects producing an increased concentration of haemoglobin in the cells, leaving that outside virtually unchanged. Hence, although Spiro's and Brinkman & Szent-Györgyi's claims were untrue in respect to the re-entry of haemoglobin into the cells, their experiments showed that the process of hypotonic haemolysis is reversible to the extent that the membrane may apparently resume its impermeability to haemoglobin after it has lost it. It is interesting to enquire how far the process of haemolysis is reversible in this case. Fricke & Curtis (1935) showed that the erythrocytes of the rabbit exhibit very similar electrical impedance both before and after hypotonic haemolysis, suggesting that the normal impermeability to cations, which is the most characteristic property of the erythrocyte, may be maintained, at least in part, after the escape of haemoglobin. The observation of Ponder & Marsland (1935) that during osmotic haemolysis the pigment may escape from the cell at a rate very much less than that required for diffusion through an aqueous medium indicates further that during the actual escape of haemoglobin the red cell membrane

still presents certain structural impediments to the free passage of this molecule. Further evidence of the at least partial maintenance of the original structure is provided by the microscopical appearance of the ghost after "reversal of haemolysis" i.e. after the addition of strong NaCl, for the form is more or less that of a typical bi-concave disk.

Davson & Ponder (1938) have shown, however, that the ghost has lost its power of preventing the passage of sodium and potassium: in fact the exchanges of these ions, which Davson & Ponder measured chemically, were so rapid that equilibrium had been established before there was time to separate the ghosts from their suspension medium at 0°. We may envisage three possibilities regarding the mechanism of hypotonic haemolysis:

- 1 The cell is ruptured into a number of fragments.
- 2 The cell membrane becomes stretched, so that pores already present are enlarged to allow the passage of haemoglobin.
- 3 The cell membrane breaks, one or more large holes appearing.

Possibility 1 is ruled out by the fact of the reversal of haemolysis, and 2 is also excluded, since to produce a shrinkage of the cell necessary to bring about reversal, a large portion of the membrane must remain impermeable to salts; this would not be the case were pores over the whole membrane stretched to the extent that haemoglobin could pass through them. Such a view would also not be consistent with the comparatively long "fading times", i.e. the times required for about 90% of the haemoglobin in a cell to escape, obtained by Davson & Ponder for osmotic haemolysis. By exclusion we are left with hypothesis (3), which is quite consonant with the known facts, and allows of a permeability of the ghost to cations through the large hole or holes, whilst leaving the cell with a net electrical impedance due to cation impermeability over the rest of the surface.

The general picture we may deduce for the process of osmotic haemolysis is, then, a swelling up of the cell until it can no longer contain any more water; when this point is reached, the membrane breaks, perhaps leaving one large rent or several smaller holes; the size of the holes is, however, large compared with the haemoglobin molecule. As a result, haemoglobin and the salts in the erythrocyte leak out, and the rate of leakage or the "fading time"

will be proportional to the size of the hole, i.e. the fraction of the cell surface available for diffusion. The ghost is now in osmotic equilibrium with its environment. Addition of strong NaCl solution will now raise the osmotic pressure outside the ghost, and this difference will be neutralised *a* by the passage of salts into

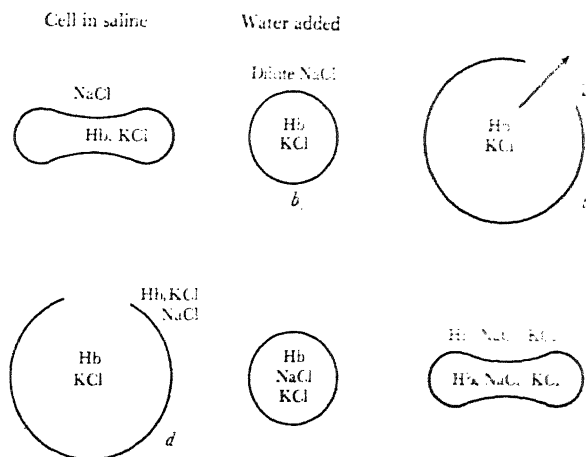


FIG. 57. Diagram illustrating haemolysis and the "reversal" of haemolysis.

the ghost through the hole, and *b* by the passage of water out of the ghost over its whole surface. Since *b* will occur more rapidly than (*a*), the ghost shrinks, thereby closing up the hole and preventing the escape of haemoglobin, but the closure is not adequate to prevent exchanges of cations. These processes are portrayed in Fig. 57.

We may now enquire into the changes which occur in the erythrocyte before osmotic haemolysis begins. The normal form of the erythrocyte as it occurs in the blood stream is that of a bi-concave disk; Fig. 58 is the mean outline of the human erythrocyte viewed end-on, constructed by Ponder (1934*a*) from his own measurements. It is clear that a cell having the shape of



FIG. 58. Cross-section of the erythrocyte, viewed end-on.

It is clear that a cell having the shape of

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a flat disk may increase in volume by transforming itself into a sphere without any change in surface area; however, once the spherical form has been reached, further increase in volume can only be achieved by an increase in the area of the membrane, either by stretching or a replenishment from the interior. This transformation into a sphere is a matter of common observation, but the extent to which an erythrocyte can increase further in volume was not known until the ingenious papers of Ponder

(1937) and of Castle & Daland (1937) appeared independently. These investigators have shown that the point at which a cell haemolyses is virtually when it becomes a sphere; in other words, there is practically no change in the surface area as it swells up and haemolyses.

TABLE LXI

I. Hypotonicity	II. Serum concentrations	III. Saponin
Goat	Cat 0.174 <i>M</i>	Guinea-pig
Ox	Ox 0.166 <i>M</i>	White rat
Cat	Sheep 0.165 <i>M</i>	Dog
Grey	White rat 0.162 <i>M</i>	Grey rat
Pig		Rabbit
	Goat	Pig
	Man 0.15	Grey mouse
White rat		Cat
Rabbit		Ox
Guinea-pig		Goat
		Sheep

It has been shown in Chapter III that the cells in any given suspension of erythrocytes undergo haemolysis at varying degrees of hypotonicity; thus only about 10% of ox cells haemolyse in 0.10 *M* NaCl, whilst about 80% haemolyse in 0.08 *M* NaCl; again, if different species are compared, one may obtain a series of increasing resistance to hypotonic haemolysis as in Table LXI, column I (Rywsch, 1907). The cause for the variable resistance to hypotonicity, or the variable "fragility", as the reciprocal of the resistance is called, has not been adequately explained. It seems fairly definite that fragility has nothing to do with the actual weakness of the membrane, for it is far too thin to exert any appreciable resistance to osmotic forces. A factor of importance will obviously be the concentration of osmotically active materials normally in the cell; thus, *ceteris paribus*, a cell with a high internal salt concentration will haemolyse at a higher tonicity than one with a lower. This point was investigated by Benham & Davson

unpublished, and in Table LXI, column II, is presented the series of animals obtained by arranging them in the order of decreasing concentration of osmotic materials in their sera (and presumably in their cells). There is no apparent correlation between the series obtained in this way and that of Rywosch, so that the concentration of osmotic materials is not the determining factor; nevertheless, it may be sufficiently important to obscure other correlations. Gansslen (1922, and later Haden 1934, 1935) have made the suggestion that the decisive factor is the length/thickness ratio of the erythrocyte, since this factor will be proportional to the extent to which an erythrocyte can take up water. Such a suggestion agrees well with the results of Ponder and of Castle & Daland, quoted above. Unfortunately the experimental values for the lengths and thicknesses of cells are uncertain, but Ponder (1937) provides reliable figures for the mean length/minimum thickness ratios of the man, rabbit and sheep erythrocytes, being 8.4, 7.3 and 5.2, which is the order of decreasing resistance to hypotonicity. However, as Ponder (1937) points out, a different order would be obtained were the length/greatest thickness chosen, and the cell shape cannot be defined in terms of a single ratio; nevertheless, Gansslen's hypothesis is probably right in its essentials.

Brinkman & Van Dam (1920) claim that washing erythrocytes with saline solution increases the osmotic resistance, and further, that the cause of this increase in resistance is the removal of lecithin, which is responsible, so they say, for the sensitivity of the cell to hypotonicity. Their experimental results are circumstantially described, and it is therefore all the more surprising that Saslow (1932) has been unable to confirm any of their claims. The removal of lecithin from the erythrocyte (presumably from its membrane) would be an interesting fact if somewhat difficult to accept.

A point of interest in connection with the problem of the changes in the membrane leading to haemolysis is raised by the work of Davson (1937), who has shown that the erythrocytes of all of six species examined become permeable to potassium when the cell swells, yet the point on the swelling curve at which this permeability occurs is considerably removed from the point at which they become spheres. If we accept the evidence of Ponder and of Castle & Daland, which shows that no measurable stretching of

the membrane occurs until the cell becomes a sphere, it is clear that the re-orientation of the membrane itself, as the cell assumes intermediate forms between a bi-concave disk and a sphere, is sufficient to produce changes in permeability; these changes are, however, reversible, so that causing the cells to shrink prevents further potassium escape.

Haemolysis by Lysins. There is a large variety of reagents which cause haemolysis of the erythrocyte, and the requirements of a lysin seem to be that it should be either surface active, e.g. saponin, digitonin, or taurocholate, or that it should be a lipoid solvent, e.g. ether, chloroform, etc. The lysin most studied has been saponin, a glucoside, and attention will be confined mainly to this substance. Ponder (for a full description see his monograph, 1934*a*) has derived an equation relating the time required to produce complete lysis and the dilution of the saponin required to produce the lysis, based on the assumption that the time elapsing between the addition of the lysin to the system and the resulting lysis of the cells is determined by an n th order reaction of saponin with the membrane. It is difficult to assess the value of this work so far as the elucidation of the actual haemolytic process is concerned, since the final equation derived by Ponder to describe the time-dilution curve contains a parameter of indefinite meaning, i.e. the exponent, n , in the fundamental equation should be an integer if it is to have any simple dynamic meaning. In actuality the exponent is not an integer, so that a fit of the theoretical and experimental curves does not necessarily mean that the fundamental assumption is true. Studies on the rate of disappearance of the lysin from a suspension of ghosts

Ponder, 1934*b*, 1935 also strongly indicate that matters are more complicated than the simple equation would indicate, and Ponder himself is inclined to substitute the idea of a varying velocity constant for the idea of an n th order reaction. Davson & Danielli (1938) and Davson & Ponder (1940) have suggested that an element in the haemolytic process may be the permeability to cations induced by the haemolytic agent which would allow of a secondary Donnan swelling of the cell; if this secondary process is of importance, then the time required for haemolysis will be a function of the time required for the reaction of the lysin with the membrane and the time required for the Donnan swelling to proceed. Nevertheless, the final equation derived by Ponder

provides excellent theoretical values for the time-dilution curves of many haemolytic systems, once the parameters have been determined, so that a constant, x , the quantity of lysin used up to produce complete lysis of the cells under any set of conditions, may be used as an excellent measure of the activity of the lysin, or alternatively of the resistance of different cells to a given lysin. The effect of temperature on all the constants in Ponder's equation is, however, quite unpredictable, and cannot be expressed in any simple way (Ponder & Yeager, 1930; Gordon, 1932).

TABLE LXII. COMPARISON OF THE RESISTANCE OF CELLS OF DIFFERENT SPECIES TO SAPONIN AND HYPOTONICITY. PONDER, SASLOW & YEAGER, 1930.

Animal	Resistance to saponin	Resistance to saline
Marmoset	0.57	0.40
Baboon	0.59	0.18
Lemur	0.64	0.42
Squirrel	0.90	0.27
Green monkey	0.90	0.43
Man	1.00	0.32
Opossum	1.10	0.35
Elephant	1.50	0.28
Chimpanzee	1.55	0.22
Armadillo	2.10	0.31
Skunk	3.7	0.39
Buffalo	7.0	0.42
Elk	8.0	0.42

Various attempts have been made to correlate the behaviour of the erythrocyte in respect to saponin with its sensitivity to hypotonic haemolysis, and Rywosch (1907) gave the series shown in Table LXI, column III, for the resistance of the erythrocytes of different species to saponin, the most resistant species occurring first, as in column I. Rywosch pointed out that the orders of the species are in the opposite sense, the most resistant to saponin being the most sensitive to hypotonicity, and it was supposed that there is a single factor in the membrane which determines the resistance to hypotonicity and saponin, this factor acting in opposite senses in the two cases. Ponder *et al.* (1930), by extending Rywosch's investigations to twenty other mammals, found no clear relation between the resistance to saponin and to hypotonicity, as may be seen from Table LXII, where some of the species not studied by Rywosch are tabulated. It thus seems that the inverse relation obtained by Rywosch was the result of the

accidental choice of mammals which happened to show this relation, and the single factor postulated by Rywosch is probably non-existent.

Port (1910) pointed out that the series of resistance to saponin is the same as the series obtained from the phosphoric acid content of the cells, the most resistant to saponin having the smallest phosphoric acid content; other suggestions have been made in regard to the cholesterol content (Yagi, 1911; Fabre & Simonnet, 1926) and the cholesterol lecithin ratio (Brinkman & Van Dam, 1920). The most recent work of Ponder and his collaborators, and of Kofler & Lazar (1927), shows, however, that little or nothing is to be derived from these series of species, since every lysin studied gives a new series.

The formation of a ghost after hypotonic haemolysis has already been described; from an erythrocyte suspension, after haemolysis with saponin, taurocholate, chloroform, etc., ghosts may also be obtained by centrifuging. The ghosts differ, however, from hypotonic ghosts by not becoming impermeable to haemoglobin on addition of concentrated NaCl solution; this may be due to either or both of two causes: *a* the membrane on treatment with

has become permeable to cations over the whole surface, so that no osmotic shrinking can be brought about, or (*b*) the damage caused by saponin is too great to allow of the repair of the membrane by shrinkage, as occurs, apparently, with hypotonic ghosts. Ponder & Marsland (1935) have studied the fading times during haemolysis with different concentrations of saponin; the results are shown in Fig. 59, and they indicate that the rate of escape of haemoglobin increases rapidly with the strength of saponin solution used to produce haemolysis. The best interpretation of these results, having regard to the long fading times at low dilutions, is that saponin attacks certain spots on the membrane preferentially and, at these spots, holes are formed; the greater the concentration of saponin, the greater the probability that the holes formed at the instant of observable escape of haemoglobin will be large and/or numerous, and consequently the shorter the time required for the escape of haemoglobin. The haemolysis produced by saponin will hence be different from hypotonic haemolysis in that a portion of the membrane may actually be lost by the hole formation; hence a shrinkage by hypertonicity cannot close the gap in the membrane and thus

"reversal" of haemolysis will not be observable. Fricke & Curtis (1935) have been unable to find any appreciable change in the electrical impedance of erythrocytes undergoing saponin haemolysis with moderate concentrations of the lysin, a fact which conforms well with the idea of one or more holes in the membrane, the rest of which is intact so far as its cation permeability is concerned. Furthermore, Ponder & Neurath (1938) have shown

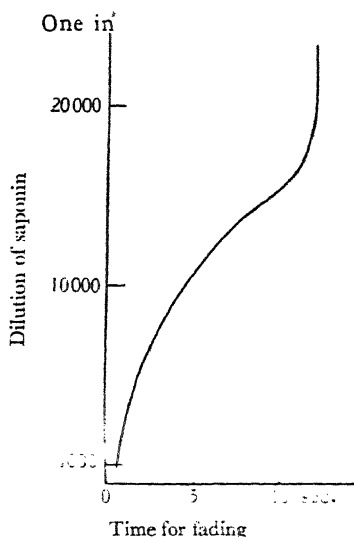


FIG. 59. Effect of variation in saponin concentration on the "fading time" of erythrocytes.

that the amount of lysin used up by a cell suspension is, when the dilution of the saponin is high, insufficient to provide a layer one molecule thick over the whole cell surface, a fact which obviously indicates a localised action of the lysin; these authors discuss their results in the light of a possible holing of the membrane, and their paper should be consulted for a more complete discussion than can be given here. The failure of Abramson *et al.* 1938, to observe any change in the electro-cataphoretic velocity of the erythrocyte after saponin lysis would further indicate that large areas of the erythrocyte surface are relatively unaffected by lytic action; however, the interpretation of electrokinetic studies is difficult.

It is not intended in this treatment of the phenomena of lysis to give a complete catalogue of the various ways of inducing haemolysis; however, there is a group of substances, known as photo-dynamic lysins, which present an interesting feature, the clearing up of which may lead to important deductions regarding the more general phenomena of haemolysis. Photo-dynamic haemolysis is caused by the action of visible light in the presence of a dye of the fluorescein class, e.g. eosin or rose bengal. The effects of these dyes were first described by Tappeiner & Jodlbauer (1904). Schmidt & Norman (1922) have shown that the haemolysis caused in this way is dependent on an oxidative process and may be inhibited by reducing agents. Blum and his collaborators (1937-1940) have confirmed this interpretation, and characterise the function of the dye stuff as a photo-sensitiser which is adsorbed on the surface of the erythrocyte and activates oxygen molecules in the close vicinity, which latter react with some constituent of the cell membrane. Blum's work also shows—and this point is the interesting feature to which we have referred above—that the changes induced in the cell on illumination do not manifest themselves immediately, since after cutting off the illumination when a definite amount of haemolysis has occurred, or even when no haemolysis has taken place (Davson & Ponder, 1940), an increasing amount of haemolysis is observed during several hours subsequently to the irradiation.

Davson & Ponder (1940) have investigated this "after-light" phase of activity and have shown that, unlike the illumination phase, it cannot be inhibited by reducing agents, e.g. sulphite, so that its cause is not due to the persistence of the oxidative chemical reaction initiated in the light phase. On the basis of studies of the photo-dynamically induced cation permeability which precedes haemolysis, and the fading times of cells undergoing photo-dynamic haemolysis, Davson & Ponder conclude that the "after-light" haemolysis is due to an osmotic swelling following damage of the membrane during illumination. The delayed action of the photo-dynamic reaction, whereby haemolysis proceeds for some time after the reaction has ceased, is apparently a unique feature of this class of lysins; Ponder & Davson (unpublished) have provided evidence that the reaction of saponin with the membrane runs hand in hand with the haemolysis produced, so that an alteration in the experimental conditions

during the course of saponin haemolysis has an immediate effect, and there is no "carry-over" from the original conditions.

The Effect of Accelerators. Ponder (1939) and Ponder & Hyman (1939) have studied the influence of certain organic substances, themselves not present in lytic concentrations, on the lysis produced by saponin and taurocholate; some of these substances have a remarkable accelerating action on the rate of haemolysis. The degree of acceleration is measured in terms of a factor $(R-1)/c$, where R is the ratio of the concentrations of the lysin required to produce complete lysis in a given time in the absence and in the presence of the accelerator. In Table LXIII values of

TABLE LXIII. THE EFFECT OF SUBSTITUTED BENZENE DERIVATIVES ON SAPONIN HAEMOLYSIS (PONDER, 1939)

$(R-1)/c$ is a measure of the acceleration; c_{\max} represents the solubility of the derivatives in water

Substance tested	mM. per l.	$R-1$
C_6H_6	10.2	-0.02
C_6H_5Cl	1.0	-0.18
C_6H_5Br	0.5	-0.20
C_6H_5I	0.5	-0.62
<i>o</i> - $C_6H_4Cl_2$	0.5	-0.78
<i>m</i> - $C_6H_4Cl_2$	0.5	-0.70
<i>p</i> - $C_6H_4Cl_2$	0.4	-0.65
<i>p</i> - $C_6H_4Br_2$	0.1	-2.2
<i>p</i> - $C_6H_4I_2$	0.02	-3.9
1-2-4- $C_6H_3Cl_3$	0.10	-1.5

$(R-1)/c$ are given for benzene and a number of its halogen derivatives; it is clear that iodine is more effective than bromine and chlorine in increasing the activity of benzene; *p*-diiodobenzene has a remarkably strong effect. Calculations made by Ponder indicate that the accelerator may exert appreciable action without necessarily covering the whole of the erythrocyte membrane, a point which apparently conforms with the notion of a localised action of the lysin. A further interesting point brought out by Ponder's work is that the reversibility of the effect of the accelerator depends on the time during which it is in contact with the cells, so that if cells are in contact with benzene, for example, for as long as 2 hours, the resistance of the cells to saponin is permanently reduced. Ponder's conception of the action of an accelerator is that the latter penetrates the membrane and, by reaction with it, weakens it in some way so that the subsequent action of the

lysin is facilitated. If this is the case, a given substance will be a more efficient accelerator the more it is concentrated in the oil of an oil-water interface, i.e. the higher its oil-water partition coefficient. The large differences in effectiveness of the substances examined may then be due to the large differences which will probably exist in regard to their oil-water partition coefficients; a better index of accelerator activity would hence be given by dividing $R-1$ by c' , the concentration of the substance in the membrane, as opposed to the bulk concentration. Apparently, however, the difficulties in determining partition coefficients of many of the compounds studied are too great to be surmounted; this point is adequately discussed by Ponder (1934*a*, pp. 181-4).

This brief review of the main experimental facts in the field of haemolysis reveals that this branch of the study of cell membranes is still poorly developed, so that there are few results which can give direct information regarding membrane structure. The results indicate that the erythrocyte membrane is not homogeneous, since there are apparently spots which are preferentially attacked by lysins, with the result that holes in the membrane occur: this evidence is in conformity with the deductions of Danielli derived exclusively from analysis of the results on permeability studies (Chapter VIII). The main problem of how the lysin causes these holes, or structural infirmities, has not apparently been seriously attempted. The old notion that lysins were always lipid solvents and that consequently lysis could be regarded as a dissolving of parts of the membrane is obviously inadequate; however, the converse generalisation, namely that all strongly lipid soluble substances are lysins, apparently holds, so that a weakening of the lipid constituents of the membrane is obviously a factor. Schulman & Rideal (1937) have shown that the extent to which a substance may penetrate a complex lipid-protein film is strongly correlated with its power of haemolysis, hence it would seem reasonable that in order to exert a haemolytic effect certain substances may have to penetrate the lipid constituents of the membrane; the stability of the mixed films so obtained will depend on the nature of the substance and its concentration; Schulman & Rideal's work would indicate that with many substances an increased stability is first obtained, but the strong correlation between penetrability into a mixed lipid-protein film and haemolytic power indicates that at least above a limiting

concentration, penetration would lead to the destruction of such a film. The fact that saponin was found not to penetrate such a lipid-protein film is, however, a point which requires further investigation, since saponin is a very strong lysin. Since heavy metals and protein precipitants may cause haemolysis, it appears that a substance which acts directly on the protein constituents of the membrane may also be haemolytic; whether or not one may separate the action of lysins in this way is, however, doubtful. Future research on haemolysis would apparently most probably be carried out by a development of the work of Schulman & Rideal, together with a vigorous attack on the nature of the interaction of substances like saponin and soaps with the membrane constituents. The fundamental conception behind Ponder's work is that the reaction is comparable with a simple chemical reaction; this conception has not been fruitful with regard to results in the experimental field and a more physico-chemical basis for the interaction should be considered.

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CHAPTER XIX

MEMBRANE PERMEABILITY IN RELATION TO SECRETION

By J. F. DANILLI

IT is not intended here to discuss the whole field of secretory activity. Such discussion, in view of the present development of the subject, would be premature. We shall discuss certain mechanisms of secretion which have been proposed in relation to the demands which these mechanisms make upon membrane properties.

Secretion of Water. It is generally assumed that if two solutions of different tonicities are separated by a membrane permeable to water, then water will flow from the solution of lower to the solution of higher osmotic pressure until the two solutions reach the same osmotic pressure. This assumption is accurate only when applied to equilibrium conditions, and frequently, even with an inanimate membrane such as a dead pig's bladder extracted with ether, the initial movement of water is in the opposite direction to that predicted by considering only the concentration gradient.

In view of this it has been suggested that here is a possible mechanism by which water may be removed from, say, the glomerular fluid by the tubules of the kidney, resulting in the secretion of a hypertonic urine. The hypertonic fluid issuing from the tubule could be a product simply of an initial transient mechanical flow of water into the fluid of the tubule cells, etc., and if the fluid were left sufficiently long in the tubule the direction of water movement would be reversed, with formation of a urine of the composition demanded by thermodynamics.

This suggestion, however, is based on a misunderstanding of the nature of anomalous osmosis. For whilst in anomalous osmosis the *volume* of the more concentrated side diminishes, so also does its *concentration*, whilst the volume and concentration of the more dilute side both increase. Table LXIV shows an example of this, taken from Schreinemakers (1938); oxalic acid (solution *B*) is separated from water (*A*), kept pure by constant flow, by a pig's

bladder. It will be seen that the *volume* of the concentrated side, i.e. the side containing oxalic acid, diminishes continuously from the beginning of the experiment; but the *concentration* on this side also decreases. If the membrane had been cellophane the direction of flow of the water would have been reversed, i.e. the volume of the more concentrated solution increases.

TABLE LXIV. DIFFUSION OF WATER AND OXALIC ACID AT 20° C. IN THE SYSTEM:

A WATER PIG'S BLADDER MEMBRANE B (8.08% OXALIC ACID)

The figures are for the amounts diffusing between successive determinations

Time hours	Diffused B to A		% oxalic acid in B
	gm. oxalic acid	gm. water	
0	0	0	8.08
3	1.57	0.89	7.70
9	2.20	2.58	7.19
14.5	1.95	2.54	6.72
21	2.13	3.24	6.20
30.5	2.82	4.99	5.48
39.5	2.62	4.94	4.78
58	4.49	9.92	3.47
80	3.95	11.54	2.20
103	2.61	10.56	1.25
126.5	1.51	7.36	0.60
168	0.59	6.08	0.11
190	0.12	1.68	0.03

Since, then, even in anomalous diffusion the net effect is always to decrease the concentration of the fluid of higher concentration, anomalous diffusion does not suffice to account for the production of a hypertonic urine.

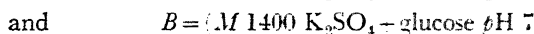
One possibility, however, remains. Returning to the experiment with a pig's bladder given in Table LXIV, we find that the net composition of the fluid which flows from the more concentrated to the less concentrated solution is usually much greater than the concentration of the solution from which it flows. For example, between the third and ninth hours, movement from B→A occurs from a solution of initial concentration 7.7%, final concentration 7.19% oxalic acid. But the net composition of the material transferred from B→A is 2.2 gm. oxalic acid, 2.58 gm. water, i.e. about 45% oxalic acid. Hence, if some means could be devised of trapping this net outgoing fluid, we should obtain a "secreted" fluid of seven times the concentration of the original fluid. So far no such scheme has been devised.

Salt Accumulation. Many cells will accumulate salt in their interior to a concentration far exceeding that in the normal environment of the cell. In some cases the osmotic pressure in the cell is equal to that of the environment, but certain ions are present in the cell to the partial or complete exclusion of other ions present in the environment. In other cases the total intracellular osmotic pressure may be greatly in excess of that in the environment. Into the first category fall cells such as those of muscle, which maintain a K^+ concentration of about twenty times or more that of the normal environment. Into the second, such cells as *Nitella* protoplasts. To explain this a considerable number of hypotheses have been put forward, e.g. by Teorell, 1935; Netter, 1928; Brooks, 1929; Osterhout, 1933; Briggs, 1930; Hartley, 1937 and Conway & Boyle, 1939.

Of these we may consider those of Netter, Teorell, Conway & Boyle and Hartley as typical. Netter pointed out that if we take a membrane permeable only to cations, e.g. a dry collodion membrane, separating solutions containing e.g. H^+ and K^+ ions, at equilibrium we should find

$$\frac{[H^+]_A}{[H^+]_B} = \frac{[K^+]_A}{[K^+]_B},$$

where A and B refer to the two sides of the membrane. This follows from the Gibbs-Donnan equilibrium. Hence if $[H^+]_A$ is greater than $[H^+]_B$, then $[K^+]_A$ must be greater than $[K^+]_B$. For example, Netter took for initial solutions



the glucose being added to equalise the osmotic pressures. After 13 days he found the following changes:

	Initial	Final
for potassium,	$\frac{[K^+]_A}{[K^+]_B} = 1$	$\frac{[K^+]_A}{[K^+]_B} > 25$
for hydrogen ion,	$\frac{[H^+]_A}{[H^+]_B} = 10^8$	$\frac{[H^+]_A}{[H^+]_B} = 52$

so that there had been a "secretion", or concentration ratio of K^+ established in A relative to B , of 25-fold—at the expense of loss of H^+ . The only demands made upon the *membrane* in this

experiment are that it should be permeable to H^+ and K^+ , but not to anions. Other writers have pointed out that if there is a continuous production of an acid inside the "cell" it is not necessary that the membrane shall be impermeable to anions. Consider the following example after Teorell, illustrated by Fig. 60. In this diagram the concentration of KBr on side B is kept constant and the $[HCl]$ in A is kept constant. The membrane is permeable to all the ionic species. Then, since H^+ diffuses faster

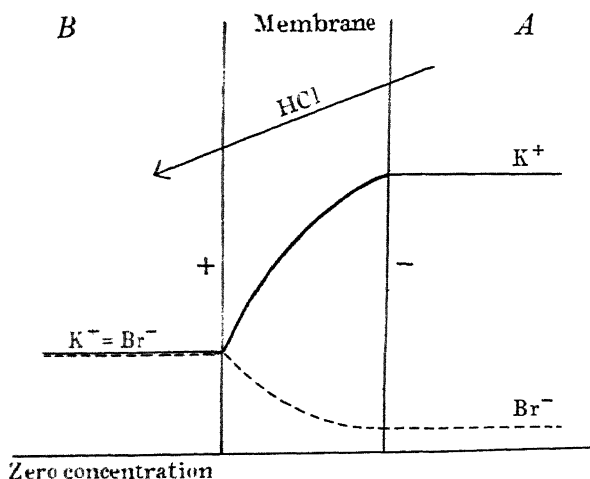


FIG. 60. The concentrating of K^+ on one side of a membrane due to continuous production of HCl on that side.

than Cl^- , and K^+ , Cl^- and Br^- diffuse at about the same rate, a potential will be set up across the membrane due to the rapidly moving H^+ ions outstripping the Cl^- ions; this potential will not be neutralised by the K^+ ions. Hence the side A will become negative, so that K^+ ions will be attracted and Cl^- ions repelled. This will result in an accumulation of K^+ ions inside the cell, and we should eventually find $[K][Cl]_{\text{inside}} = [K][Cl]_{\text{outside}}$. Since the K^+ not neutralised by Br^- will be neutralised by Cl^- left in A by the rapidly diffusing H^+ , the excess of H^+ over Cl^- to diffuse through the membrane will be neutralised by the Br^- which has been prevented from accompanying the K^+ into the interior of the membrane by the diffusion potential.

Hartley (1937) has suggested a mechanism combining the actions of several membranes by which a salt solution may be concentrated at the expense of dilution of a second substance, continuously produced by metabolism. Consider the system bounded by the three membranes *A*, *A'* and *B*. Fig. 61. *A* and *A'* are membranes only in the sense that they prevent convection, and are permeable to all substances. Membrane *B* is permeable, in the simplest case, to water only. A diffusible product is produced in the compartment 2, and if produced in sufficient concentration water will flow from 1, to 2. Consequently there will be a net flow of water from *A* to *A'*, as shown by the long arrow. Salts will accumulate in (1) to an extent determined by

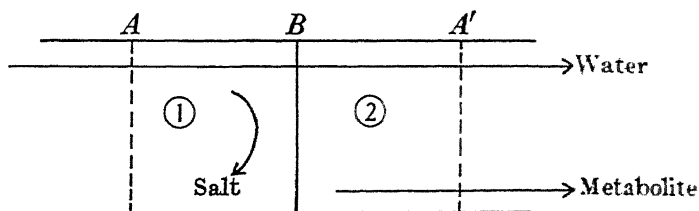


FIG. 61. Diagram to illustrate the concentrating of a salt solution. A diffusible metabolite is continuously produced in (2) and is diluted by water flowing from (1), so that the salt solution in (1) becomes more concentrated.

the rate of production of the diffusible metabolite, the permeability of *B* and the rate of back diffusion through *A*. The water removed from (1) will move out of *A'* together with the metabolite. As Hartley points out, accumulation by such a process cannot occur with only one type of membrane, such as either *A* or *B*, but with two types accumulation can be achieved very simply. Gaddum (1937) has suggested that this model of Hartley's is to some extent comparable to a kidney tubule, (1) being the lumen of the tubule, and (2) a tubule epithelial cell.

Finally, we may consider the proposals recently put forward for the concentrating of K^+ in muscle by Conway & Boyle (1939). They assume that the membrane of a muscle cell is permeable to H^+ , K^+ and small anions, but not to other cations. Anions, such as those of phosphate and amino acids, diffuse through the membrane accompanied by K^+ . The amino acids, etc. are synthesised into proteins and other non-diffusible anions, so that

ultimately a large amount of anion is fixed inside the cell, and consequently a large amount of K^+ is electrostatically "bound", i.e. prevented from diffusing out of the cell. The resting potential of muscle is therefore due to the "attempt" of the K^+ ions to diffuse out and to the restraint exercised on them by the non-diffusible negative ions. This is a very promising theory.

All these various suggestions for ion secretion make demands on the membranes of cells which are, up to a point, plausible, and may be reproduced within reason in experiments on models. But it has yet to be shown that any actual living system does in fact secrete ions in this way. Many facts suggest that the phenomena are more complex than is supposed in such theories. For example, the resting potential of nerve and muscle is to some extent linked with metabolism. If, for instance, a nerve is put into an atmosphere of nitrogen, the resting potential falls off, but this is not due to an equalisation of K^+ concentrations on the two sides of the membrane—in fact practically all the K^+ remains in the cell and the potential may be restored by admission of O_2 (Cowan, 1934). Then again, when muscle is stimulated some K^+ leaves the cell and some Na^+ enters. According to Conway & Boyle, the K^+ loss is due to formation of diffusible anions (lactate, PO_4 , etc.) and the K^+ is eventually restored to the muscle in the same manner as its initial concentration arose. But this does not account for the fact that the Na^+ which has entered is ultimately expelled again.

Such facts as these lead to the conclusion that, although there may be a large measure of truth in some of these proposals, they none of them correspond very closely with *all* the biological facts. The direct demands these theories make on membrane permeability are simple and reasonable, but in actual fact the permeability of the natural membranes which secrete ions is complicated to a degree which has not yet yielded to analysis.

Secretion of Sugars. There are two, possibly separable, phenomena which need explanation: (1) certain sugars are able to penetrate, e.g. the intestinal mucosa and the kidney tubule, much more rapidly than others, in the absence of certain poisons; (2) certain sugars may be completely removed from e.g. glomerular fluid in the tubules, if phosphorylating reactions are not interfered with.

Wilbrandt & Laszt (1933) and other workers have inferred that this acceleration of diffusion and removal of sugars is dependent

on phosphorylation of the migrating sugar molecules. Verzar & McDougal conclude 1936 that "the selective absorption of glucose (and galactose) is a phosphorylation of these sugars and acts by increasing the diffusion gradient into the mucosal cell". This, however, is an over-simplification, for phosphorylation alone can make no significant difference to the rate of penetration. Consider Fig. 62 *a*; it represents diffusion of a sugar across a mucosa cell—the outer membrane exposed to the intestinal contents is *A*, and the inner membrane exposed to the body fluids is *B*. We will assume that the area of *A* is equal to the area of *B*, and that both *A* and *B* are permeable to sugar. Then the initial concentrations for a sugar which penetrates without phosphorylation will be those represented by Fig. 62 *a*, and the final concentrations those of Fig. 62 *b*. In the final state there would be equal concentrations of the sugar in the intestine and in the body fluids. Now consider the simple case of a phosphorylating mechanism, which converts the greater part of the sugar which enters the cell into ester (Fig. 62 *c*): this diffuses across the cell to *B*, and in passing through *B* into the body fluids is converted back to sugar, presumably by an enzyme associated with the cell membrane *B*. From the point of view of the absorbing process, the efficiency of secretion is given by the amount of sugar passing through *B*, and this will ultimately be proportional to the total concentration of potential sugar, i.e. sugar + ester just inside *A*. The question is, therefore, Does phosphorylation increase the concentration of potential sugar at *A*? Now for a given concentration, *C*, of sugar in the intestine, the amount of sugar entering the cell per unit time is αC , where α is a constant. When no phosphorylation occurs (Fig. 62 *a*) the amount leaving the cell and diffusing back into the intestine is $\alpha C'$, where C' is the concentration of sugar just inside *A*. When the sugar is phosphorylated $C' = 0$ approximately, so no sugar diffuses back into the intestine. But potential sugar may, for ester will diffuse back into the intestine at the rate $\beta C''$, where C'' is the concentration of ester just inside *A*. In free diffusion the rates of diffusion of sugar and ester are practically the same (actually the ester is rather slower), so that α is approximately equal to β , i.e. the loss of potential sugar by back diffusion into the intestine is essentially the same, whether the sugar is present inside the cell as ester, or as free sugar. Consequently $C' = C''$, and we conclude (case i)

that phosphorylation alone will make no difference to the rate of absorption of sugar.

A quite different state of affairs arises if we assume case ii that membrane *A* is permeable to sugars, but relatively impermeable

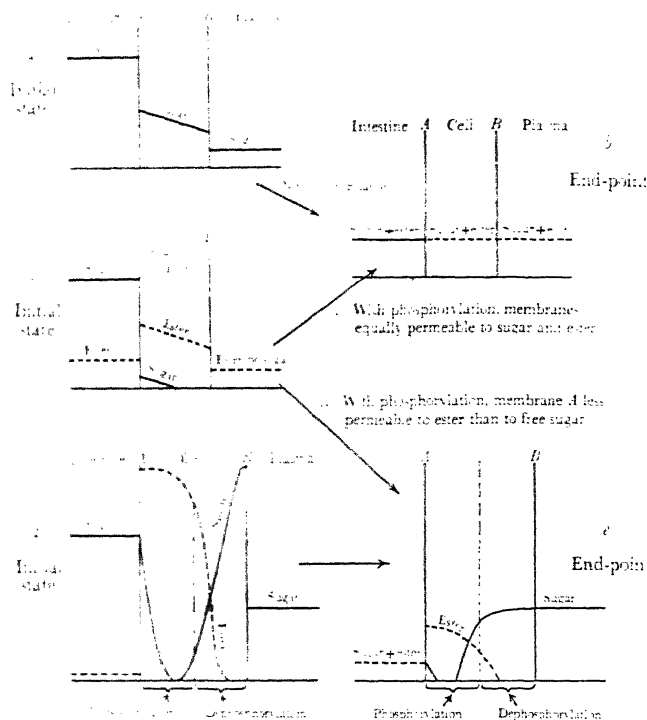


FIG. 92. Effect of phosphorylation on the diffusion of sugar. Concentrations are plotted vertically, distance horizontally. In case (a) there is no phosphorylation. In case (i) there is phosphorylation inside the cell and dephosphorylation at membrane *B*; no accumulation occurs if membranes *A* and *B* are equally permeable to sugar and to phosphorylated sugar (case i). But, (ii), if the membranes are less permeable to ester than to free sugar, accumulation occurs. (d) is an alternative version of (c), ii with both phosphorylation and dephosphorylation occurring in the cytoplasm.

to esters (i.e. $\alpha \gg \beta$). Back diffusion is eliminated, and the concentration of potential sugar will increase. If the area of *A* = area of *B*, the maximum increase in the rate can be calculated from diffusion kinetics to be a factor of 2. In practice it will lie

somewhere between 1 and 2, probably nearer to 1 than to 2 if the cell membrane has a permeability to sugars of the same magnitude as have most other cells. A further increase in the rate of absorption may be made by increasing the ratio area *A* area *B*, since the greater the value of this ratio, the higher will be the concentration of ester inside the cell. Furthermore, not only is the rate of absorption increased, but, since almost no back diffusion occurs, almost the whole of the sugar present in the intestine (or the tubule) will be removed.

Several points must be noted about this mechanism of secretion. (1) Energy is required at the phosphorylation stage only—a point where energy is usually available in cells; (2) the mechanism of secretion will not transport an unlimited amount of sugar in a given time, since the enzymes will become saturated with high concentrations of sugar; hence, at higher concentrations of sugar the rate of transport will be independent of the concentration in the intestine; (3) this type of active transport is available for all substances which, on penetration into a cell, are easily synthesised into a substance whose rate of back diffusion through the cell membrane is less than that of the original substance.

With regard to the details of structure of the secreting cell: membrane *A* may be similar to membrane *B* if the dephosphorylating enzyme is inside the cell. All that is necessary is that the cell membranes shall be less permeable to ester than to sugar and that one region of the cell shall contain the phosphorylating mechanism and another region the dephosphorylating mechanism. This localising of the phosphorylating systems may be due to their being part of the fixed structure of the cell, such as mitochondria, or to the presence of an intercellular membrane impermeable to enzymes, as shown in Fig. 62 'a'.

Secretion of Oxygen into the Swim-Bladder of Fishes. The gas which is secreted into the swim-bladder of fishes is mainly oxygen. The mechanism of secretion is generally supposed to involve the liberation in the capillary *rete mirabile* of the swim-bladder of a substance which reduces the affinity of haemoglobin for O_2 . Since CO_2 reduces the affinity of haemoglobin for O_2 , it has been suggested that CO_2 is the substance concerned. Irving & Grinnell (1939) have recently shown that the action of CO_2 on the blood of fish is much more pronounced than on human blood, actually nine times greater. In the case of the brook trout

this mechanism would be capable of filling the swim-bladder with pure oxygen at a depth of 15 feet. The only property required by membranes in this mechanism is that the wall of the swim-bladder shall be impermeable to O_2 . That this is so has been shown by Bohr, 1893. It yet remains to be shown, however, that this is the correct mechanism, and how CO_2 is produced in sufficient quantity in the correct place.

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CHAPTER XX

THE KIDNEY

By H. DAVSON

THE removal of the waste products of metabolism and of exogenous materials from the blood is the function of the filter, and consequently we may expect to find in this organ cells whose membranes are adapted to differentiate between those substances which must not be allowed to accumulate in the blood and those which at all costs must be retained. Hence it is to be expected that the specific excretory functions of the kidney will present many interesting problems to the student of cell permeability, and this is indeed the case. Various aspects of kidney function have been reviewed in recent years (Smith, 1936, 1937; Marshall, 1934; Richards, 1934; Winton, 1937; Shannon, 1939), and in this chapter nothing more will be attempted than to present an integrated picture of renal processes in so far as it will indicate, to those primarily interested in permeability, the possibilities of research, and the complexities of the system.

The functional unit of the kidney is the nephron, comprising the renal corpuscle and the renal tubule, the latter being divided into three parts, the proximal and distal convoluted tubules and the intermediate portion, the loop of Henle. The renal corpuscle may be viewed as the blind dilated extremity of the tubule, known as Bowman's capsule, in which is invaginated a bunch of capillary vessels, the glomerular tuft. From Bowman's capsule a short neck leads into a proximal convoluted tubule and this into a U-shaped portion, the descending and ascending limbs of the loop of Henle; the latter passes into a distal convoluted tubule (Fig. 63). The distal convoluted tubule, by means of a connecting tubule, passes into a collecting tubule and eventually into the ureter. The capillaries in the glomerular tuft are derived from an afferent arteriole. They show no anastomoses and are collected to form an efferent vessel; the efferent vessel in the mammalian kidney breaks up to form a new set of capillaries which this time supplies either the proximal and distal convoluted tubules, Henle's loop and connecting tubules, or all according to the position of the

THE KIDNEY

glomerulus in kidney. It should be noted that in the mammalian kidney the tubules are supplied entirely by blood which has first passed through the glomeruli.

On the vertebrates below mammals all species have a double blood supply: the afferent glomerular vessel and the renal portal system (Jourdain, 1859; Noll, 1924; Spanner, 1924). It is generally believed that the bloods mix freely but, as we shall see, the numerous experiments of Höber and his school would strongly indicate that the two circulations are functionally separate, at least under the conditions of dual perfusion of the two systems. The distal convoluted tubule in these species is supplied by the efferent glomerular vessels, and the proximal convoluted tubule by branches from the renal portal vein.

Certain species, namely the toad-fish *Opsanus tau*, and the goose-fish *Lepidus piscatorius*, are called aglomerular, since the nephron is lacking a glomerulus; in these cases the absence of the glomerulus is to be looked upon as a differentiation of the glomerular kidney to allow of adaptation to a salt-water environment (Marshall & Smith, 1930).

The generally accepted view of the process whereby the kidney is able to excrete substances from the blood is, with some important modifications, that of Cushny (1917), who viewed the process as consisting (a) of a process of ultra-filtration of the blood in the glomerulus, whereby a fluid consisting essentially of blood minus proteins is formed in Bowman's capsule, and (b) of a process of reabsorption of necessary blood constituents, salts, sugar, amino acids, etc., during the passage of this fluid down the tubule. Metabolic products such as urea, creatinine, etc. and some water are left behind and this solution finds its way to the ureter and the bladder. Within recent years evidence has been accumulating to show that the process of filtration plus selective re-absorption

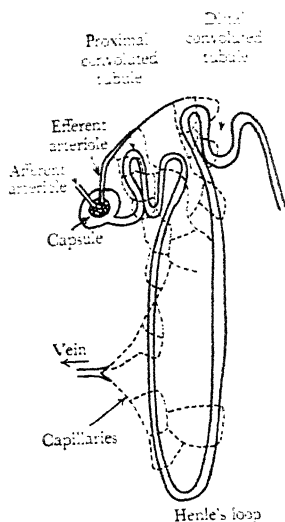


FIG. 63. Diagram of kidney structure. Part of the capillary network is indicated by the broken lines.

is not sufficient to account for all the phenomena of renal excretion, so that a third process has been invoked, namely that of tubular secretion, whereby substances are secreted directly from the blood supplying the tubules into the lumen of the latter. These three steps in the formation of urine, *glomerular filtration*, *tubular re-absorption* and *tubular secretion* will be discussed separately.

Glomerular Filtration. Glomerular filtration is essentially a filtering off of the proteins from plasma through a membrane impermeable to these but permeable to the remaining constituents of the blood; hence the factors operative in the process should be qualitatively similar to those involved in the formation of tissue fluid (Chapter III). Quantitative differences must be expected since the filtration, to be effective, must be continuous and rapid. The essential factors to be investigated in determining the fact of simple filtration in the glomerulus will be the hydrostatic pressure in the glomerular tuft—this must be high enough to allow of a rate of filtration appropriate to the urine formation—and, acting against this hydrostatic pressure, will be the colloid osmotic pressure of the plasma proteins. The effectiveness of the filtration pressure, i.e. the difference between the hydrostatic and osmotic pressures, will be influenced by the resistance to flow through the tubules.

In the frog the pressures in the glomerular tufts have been measured by Heyman (1927) on living and perfused animals; he found for living frogs a mean arterial pressure of 87.2 ± 6.6 cm. H_2O ; for the systolic afferent arterial pressure, 81.0 ± 6.6 cm. H_2O , and a glomerular capillary pressure of 29.2 ± 6.8 cm. H_2O . In only 11 out of 181 experiments did glomerular capillary pressure fall below 10 cm. H_2O , the value of the colloid osmotic pressure of the plasma proteins in the frog. In the mammalian kidney the pressure relationships have been subjected to a rather more searching analysis. Krogh (1929), and Rehberg (1935) have deduced from Landis' (1927) measurements of peripheral capillary permeability that the minimal effective filtration pressure to produce a normal flow of urine is 80 mm. Hg; if we allow a colloid osmotic pressure of 40 mm. Hg (this is the value for the most concentrated plasma which will be present in the glomerulus), and a pressure drop along the tubules of 25–80 mm. Hg, it is clear, as Winton (1937) in a frank and stimulating review points out, that the glomerular blood pressure is inadequate for its

functions if all these estimates taken together are correct. Winton discusses the influence of venous pressure, ureteral pressure, systemic arterial pressure and intra-renal pressure on the formation of urine, and shows that the minimum arterial pressure below which urine formation ceased, or alternatively the limiting ureteral pressure above which urine formation ceased, are not necessarily measures of the filtration pressures in the glomerulus.

An important aspect of the glomerular filtration hypothesis is the relative concentrations of substances in the glomerular fluid and the blood plasma. In the frog and *Necturus* the fluid in Bowman's capsule is sufficiently accessible to allow of its withdrawal, a procedure which has been successfully carried out by Richards and his school, and the fluid together with plasma or an ultra-filtrate of the same have been analysed by methods specially adapted for the small quantities of fluid obtainable.

TABLE LXV. COMPARISON OF THE CONCENTRATIONS OF CHLORIDE IN FROG PLASMA, GLOMERULAR FLUID AND BLADDER URINE. FREEMAN *et al.* 1930

Plasma	Glomerulus	Bladder	Percentage difference Glomerulus—Plasma
0.30	0.30	0.04	0
0.32	0.29	0.07	— 9.4
0.32	0.30	.	— 6.3
0.32	0.33	.	+ 3.1
0.36	0.31	.	— 13.9
0.26	0.30	.	+ 15.4
0.47	0.43	0.06	— 8.5
0.35	0.41	0.05	+ 17.1
0.42	0.47	0.008	+ 11.9
0.40	0.38	0.03	+ 5.0

In Table LXV some comparative concentrations of chloride have been selected from the paper of Freeman *et al.* (1930), and it may be observed that the concentrations, which should be approximately equal, actually differ in some cases by more than the limit of 10% imposed by the experimental errors; however, the absence of a systematic deviation in either direction with these results, and more especially with similar analyses of urea (Walker & Elsom, 1930), phenol red and indigo carmine (Richards & Walker, 1930), glucose (Walker & Reisinger, 1933), inorganic phosphate (Walker, 1933), creatinine (Bordley *et al.* 1933) and uric acid (Bordley & Richards, 1933), suggests that chemical evidence is strongly in favour of the hypothesis that the glomerular

fluid is formed by a simple process of filtration. Similarly, the equality of osmotic pressure, determined by Barger's method (Walker, 1930), and electrical conductivity (Bayliss & Walker, 1930) with those found in ultra-filtrates of frog plasma is also strong evidence of an absence of any secretory process at this stage of urine formation.

In the mammal, where the glomeruli are not accessible to puncture, more indirect methods have been used, consisting of studies of the variation of urine flow with variations in the colloid osmotic pressure of the plasma and the other physical factors enumerated earlier (Winton, 1937), and also chemical analyses of the urine in cases where tubular re-absorption and secretion of the substance analysed can be ruled out. An important piece of evidence regarding the presence of a *filter* in the process of urine formation is that provided by Bayliss *et al.* 1933, who showed that after injection into the blood of cats and rabbits only proteins with a molecular weight equal to or lower than that of haemoglobin were excreted (Table LXVI); haemoglobin was found to be excreted only when the plasma level exceeded a certain amount. Histological studies of the kidneys after the experiments revealed no evidence of kidney damage, and it was also found that the kidneys did not eliminate foreign plasma proteins, e.g. the serum albumin of an ox was not excreted by the cat.

TABLE LXVI. THE EXCRETION OF INJECTED PROTEINS BY CAT AND RABBIT AND THE ISOLATED PERFUSED KIDNEY (Bayliss *et al.* 1933)

Proteins excreted:	Molecular wt
Gelatin	35,000
Bence Jones	35,000
Egg albumin	34,500
Haemoglobin	68,000
Proteins not excreted:	
Haemoglobin	68,000
Serum albumin	67,500
Serum globulin	103,800
Edestin	208,000
Casein	188,000
Haemocyanin	5,000,000

Another line of evidence is given by the study of the *clearances* of certain compounds. The clearance is defined by the ratio $U.V/B$, where U is the concentration of the given substance in the urine, V the volume of urine formed per minute (hence UV

is the amount of substance excreted per minute' and B is the concentration in the blood. Hence the clearance represents the amount of blood which must be filtered completely to give the quantity of the substance found in the urine in 1 min. of flow, and thus could be a measure of the filtration rate if effects due to tubular re-absorption and secretion could be eliminated. With substances, or under conditions, where these two effects of the tubules may be ruled out, e.g. inulin, creatinine (in the dog, rabbit, sheep and seal or glucose (in the phlorizinised dog, dogfish, sheep and chicken), it is found that the simultaneous clearances of these three substances may be equal, a point which is best explained in accordance with a simple filtration process in the glomerulus, since it would be very unlikely that the secretion of such dissimilar substances would be quantitatively the same.

The identity of inulin clearance with glomerular filtration rate is a matter of great importance, since we have here a means of determining, under strictly physiological conditions (inulin is a polysaccharide and non-toxic), in humans and experimental animals the rate of glomerular filtration; with this definitely known the factors of tubular re-absorption and secretion can be placed on a quantitative basis. Inulin has a high molecular weight *ca.* 5000, hence its active secretion or re-absorption is unlikely *a priori* owing to the large structural changes in a cell membrane which would have to be achieved to allow of its entrance; it is completely filterable from plasma by artificial membranes and the frog's glomerulus, it is not excreted by aglomerular fishes, the plasma clearance is independent of plasma concentration, and perfusion experiments, in which glomerular filtration is excluded by the low aortic pressure, indicate that it is not secreted by the tubules of the frog, dog and rabbit.* All these facts taken together make a convincing picture of the value of inulin clearance as a measure of filtration rate.

A final argument in favour of the existence of glomerular filtration is provided by a study of the comparative physiology of glomerular and aglomerular kidneys; in the former practically all

* The evidence is summarised in detail by Smith (1937) and Richards *et al.* 1938; the credit for the important discovery of the usefulness of inulin goes to the laboratories of both Richards and of Shannon & Smith, who undertook their investigations independently.

foreign substances injected into the body are excreted, whereas in the latter this is by no means the case, e.g. during hyperglycaemia glucose is not excreted; injected ferrocyanide, creatinine, xylitol, sucrose and inulin are also not excreted.

Tubular Re-absorption. The re-absorption of salts, sugar, amino acids, etc. is a necessary consequence of the glomerular filtration theory, since otherwise the blood would be rapidly depleted of its necessary constituents; hence research has been largely directed to a study of the site of re-absorption of the various constituents and to a small extent the mechanism of this re-absorption. The

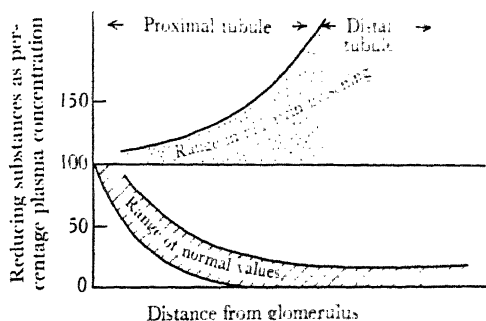


FIG. 64. Variation in glucose concentration as the glomerular filtrate passes from the glomerulus to the ureter. (RICHARDS, 1934).

micro-manipulative and chemical methods of Richards and his collaborators have lent themselves to a study of the site of re-absorption, since by this method it has been possible to measure the concentrations of various substances successively in the glomerulus, the proximal and the distal tubules. It must be remembered that the re-absorption of water will modify the concentrations of substances in the tubules and this effect must be allowed for; similarly, tubular secretion may also mask re-absorptive processes, but this is not very likely since the two processes are physiologically incompatible. In Fig. 64, taken from Richards (1934), we see the variation in the glucose concentration, as a percentage of the plasma value, as the fluid passes from the glomerulus to the ureter. In this case it is found that the re-absorption is generally complete before the proximal tubule is traversed. Perfusion of the distal tubule with a glucose-Ringer

solution indicated that glucose is not re-absorbed there. Phlorizin completely inhibits the re-absorptive process, and the increase in concentration observed in this case is due to the absorption of water. Comparison of the total molecular concentrations of substances in frog and *Necturus* glomerular and tubular fluids shows that the selective re-absorption of salts occurs in the distal tubule: as a result of this re-absorption of salts being greater than the re-absorption of water the urine of the frog is hypotonic, whereas in mammals it is found that the urine is generally hypertonic. (Comparative physiological studies of Peter, quoted by Marshall, 1934, indicate a strong correlation between the length of the thin segment of Henle's loop and the ability to secrete a concentrated urine, suggesting that this portion is the site of re-absorption of water.

We must conclude that in re-absorbing the constituents of the glomerular fluid the tubule is performing work, since we find that glucose may be *completely* re-absorbed, and this obviously indicates a transference of solute from a medium of lower to one of higher concentration. This work will be provided by the metabolic activity of the tubular epithelium cells, and consequently we may regard the action of phlorizin as a specific poisoning of the catalyst responsible for this re-absorptive process. We can, however, conceive of a re-absorption of some solutes as a simple diffusion process as a result of the concentration gradients set up by the absorption of water from the tubular fluid; in this case, of course, re-absorption will not be complete. In the phlorizinised frog glucose, xylose and creatinine clearances are equal, but they are all below that of inulin, which indicates that back diffusion of glucose, xylose definitely shown by Richards by perfusion with Ringer-sugar and creatinine by a simple diffusion process is possible, at least in the presence of phlorizin. Such back diffusion does not occur with man, dogfish, dog, chicken and chimpanzee.

It may be noted here that the action of phlorizin is apparently specific for sugar re-absorption, since the re-absorption of water chlorides and phosphate is unaffected by this substance; whilst other poisons such as CN^- and urethanes apparently block all re-absorptive processes (David, 1924, 1925).

In Fig. 65 we observe a striking rise in the concentration of urea, phosphate and glucose (in phlorizinised animals) in the distal tubule, indicating either a considerable secretory activity

on the part of the distal tubule regarding these substances or, alternatively, that re-absorption of water occurs to a larger extent here than elsewhere. The much larger concentration of urea cannot be accounted for by water absorption, as one would expect phosphate and glucose to be concentrated to the same extent, unless, of course, back diffusion occurs in actuality it will be seen later that urea is secreted by the tubules of the frog. In mammal it is observed that the clearance of urea is markedly dependent on the rate of urine flow when this is small; that is it is found that the clearance is always less than the simultaneous

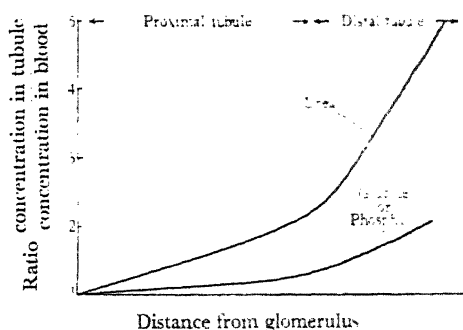


FIG. 65. The concentration of urea, glucose in the proximalised frog and inorganic phosphorus in the distal tubule of the kidney. (After Rastbach, 1934.)

inulin clearance. This latter point undoubtedly indicates a re-absorption of urea, but whether it is a simple back-diffusion process or not is difficult to say; the dependence on urine flow would certainly indicate a simple diffusion process (Shannon, 1938*a*), but the matter is complicated and has not been finally solved. In the elasmobranchs it has definitely been shown that urea is actively re-absorbed (Smith, 1936), but this is understandable since these fishes require a high blood concentration to maintain their osmotic pressure.

In mammals little is known about the re-absorption of Na^+ , K^+ , Cl^- and HCO_3^- ; it is known that they must be actively re-absorbed and further that there are probably independent mechanisms for the absorption of each of these ions, since in alkalosis HCO_3^- is excreted (i.e. re-absorbed to a smaller extent in preference to Cl^- , and in ablation of the adrenal cortex Na^+ is

excreted whilst K^+ is not: similarly, injection of KCl causes increased K^+ excretion although that of Na^+ may be unchanged. The foreign anion CNS^- is re-absorbed almost completely; similarly, Br^- and I^- ; NO_3^- and ClO_4^- are re-absorbed rather less rapidly. It may be argued, in the case of a foreign anion such as CNS^- , that as a result of the re-absorption of water in the tubules the concentrations of all these substances will be increased to such an extent that back diffusion will account for all the phenomena of re-absorption; however, with ions such as PO_4^{3-} and I^- it is known that complete re-absorption will occur only if the plasma concentration is below a certain threshold (Pitts, 1933); if the concentration rises, excretion occurs; this would be difficult to account for on the basis of a simple back-diffusion process as it would have to be assumed that minute changes in phosphate concentration changed the permeability of the tubule wall quite considerably, whereas such a sensitivity has not been found with other membranes and on the other hand it is known that enzymatic processes are very susceptible to small changes in the ionic milieu. Laug & Hober (1936), using the dual perfusion technique with the frog, showed that CNS^- is actively secreted by the tubule, Br^- is re-absorbed and I^- is neither secreted nor re-absorbed.

In the frog, phosphate is concentrated in the distal tubule (Walker & Hudson, 1937), but never more than glucose in the presence of phlorizin; in the proximal tubule these authors state that a variable degree of active re-absorption may occur; if the tubule is perfused with Ringer containing more phosphate than that present in the plasma diffusion out of the tubule occurs.

At this point some work of Hamburger & Brinkman (1918) may be mentioned. These authors perfused the kidney of the frog from the aorta and observed that with a sugar concentration of 60 mg. % a sugar-free urine could be obtained; later Hamburger (1922) showed that of a number of sugars only glucose was completely retained, whilst *l*-xylose and *d*-ribose were partially retained, i.e. there was some re-absorption of these sugars, and *d*-mannose, *d*-fructose and *l*-arabinose were completely excreted. Höber (1933) developed this work of Hamburger's and gives the following series for the relative degrees of re-absorption of the sugars:

glucose > galactose > mannose > fructose > xylose > arabinose, and points to a similar series in the intestinal absorption (Cori, 1925; Wilbrandt & Laszt, 1933). That a genuine re-absorption

was occurring in each case was shown by the action of a narcotic, phenyl urethane, addition of which to the perfusion fluid caused an increase in the sugar concentration in the urine; nevertheless, it is clear from Höber's results that the kidneys were rapidly losing their ability to re-absorb chloride in practically no instance was the urine chloride free and it was often as high as 0.5%, so that more than a qualitative significance from results of this sort of perfusion is not to be expected. An interesting point brought out by Höber was the impermeability of the tubular membrane to all these sugars in the direction blood \rightarrow tubule; to show this he made use of the dual circulation in the frog kidney whereby on perfusion of the renal portal vein access to the glomeruli is excluded; perfusion of the tubules in this way gave no evidence of the penetration of any of the sugars into the tubules; this impermeability to glucose was not affected by phlorizin. In a later paper Schmengler & Höber (1933) have studied the permeability of the tubular membrane in the direction of blood \rightarrow tubule to a number of lipoid insoluble substances with varying molecular volume and have shown that their rate of entry is apparently determined by this factor (lipoid-soluble substances had previously been shown to penetrate very rapidly: thus with amino compounds the order was roughly acetamide $>$ thiourea $>$ methylurea $>$ lactamide $>$ malonamide $>$ butyramide $>$ creatinine $>$ asparagine, the last mentioned not penetrating at all. Similarly, for hydroxy compounds the following order was obtained: glycol $>$ glycerol $>$ di-hydroxyacetone $>$ glucose $>$ mannitol, the last mentioned not penetrating).

Liang (1929), using the same perfusion technique, claimed that whereas CNS^- , K^+ and Rb^+ diffuse in the direction blood to tubule, I^- , Cl^- , SO_4^{2-} and HPO_4^{2-} do not.

An interesting demonstration of the close connection between glucose and xylose re-absorption is that of Shannon (1938), who showed that if a dog's plasma glucose level is raised to the extent that glucose appears in the urine (i.e. maximal re-absorption of glucose is being attained in the tubules. Shannon & Fisher, 1938) all the xylose injected is excreted, indicating an absence of re-absorption; it appears safe to conclude with Shannon that the absorption of glucose and xylose is mediated by the same metabolic activity and that glucose receives preferential treatment.

Tubular Secretion. The demonstration of an active secretory transport of a substance from the blood stream directly into the tubular lumen is due in the first place to Marshall & Vickers (1923) and Marshall & Crane (1924), who showed that the amount of phenol red excreted by the dog was greater than that which could be accounted for on the basis of filtration through the glomerulus and by direct chemical analysis of kidney tissue showed that urea and phenol red were concentrated in the tubule cells of the frog and only phenol red in the case of the dog and rabbit. Edwards & Marshall (1924) later proved by direct observation of the proximal tubule cells that these accumulated phenol red: they also showed that the clearance of phenol red (i.e. the amount of blood which would have to be filtered to give the amount of phenol red found in the urine in one minute) depended on the plasma concentration of this substance, increasing with increasing plasma concentration only up to a certain limit. Urea, on the other hand, showed no such behaviour in the dog, and it was consequently argued that if phenol red is excreted only by a filtration process the clearance should increase linearly with the concentration of free dye in the plasma.

Since this work, a large literature on tubular secretion is accumulating and has brought to light an amazing diversity in behaviour in regard to the substances which are secreted by the tubules in different species. Thus creatine is secreted by the glomerular and aglomerular fishes but not by mammals; creatinine by the glomerular and aglomerular fishes, by the chicken, man and the apes but not by the dog, sheep, rabbit and seal. Urea is secreted by the tubules of some fishes and the frog but not by *Necturus*, the chicken or mammals, and uric acid is secreted by birds and reptiles but is actively re-absorbed by the tubules of mammals. (For references, see Shannon, 1939.)

The dependence of the clearance of a secreted substance on the plasma concentration, mentioned above, is important, since it can lead to conclusions regarding the mechanism of tubular secretion; as a typical example of the sort of results which have been obtained some curves of Pitts (1938) are presented, Fig. 66, in which the phenol red excreted in mg. per c.c. of glomerular filtrate is plotted against the plasma phenol-red concentration. Curve *A* is the curve for the total phenol red excreted, and we observe that there is a sharp rise at low concentrations and then

an almost linear increase with a much more gradual slope. If, now, allowance is made for the phenol red excreted by filtration through the glomerulus (simultaneous determinations of inulin clearance made this possible), we get curve *C*, the phenol red secreted by the tubules, and we observe that after the rapid increase the curve becomes horizontal, indicating that there is a maximal rate of secretion which is achieved at a plasma concentration of about 5 mg. %. Curve *B* shows the phenol red filtered per c.c. of glomerular filtrate, and as we would expect this is a straight line through the origin and parallel with the

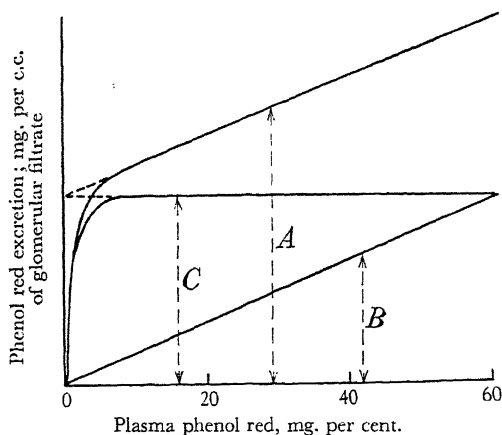


FIG. 66. Phenol-red excretion. Curve *A*, total phenol red; curve *B*, phenol red due to filtration in glomerulus; curve *C*, phenol red due to secretion by tubules.

latter part of curve *A*. As Shannon (1938*c*) has pointed out in his work on the tubular secretion of phenol red in the aglomerular fish *Lophius piscatorius*, the demonstration of a maximum rate of secretion leads to the inference, among other things, that the tubule cells have no passive permeability to phenol red in the direction of blood to tubule, so that transport of this substance is determined entirely by an active metabolic process. Shannon (1939) has set up a scheme for tubular secretion based on the following chain of reactions:

A is the solute in the interstitial fluid around the tubule cells, *B* is a cellular component with which *A* must react.

The rate of reaction $AB \rightarrow T$ determines the amount of the solute which can be transferred to the tubule lumen, i.e. it determines the rate of tubular secretion, T_s , under any conditions. This plan leads to the equation

$$K = \left(a - \frac{T}{V}\right) \left(\frac{T_s - T}{T_s}\right), \quad \dots\dots 32$$

where a is the arterial plasma concentration, T_s is the rate of tubular secretion, T_m is the maximal rate of secretion, V is the rate of flow of blood through the vessels supplying the tubules.

This equation has been applied to various systems and the theoretical curves fit the experimental ones remarkably well; however, it is difficult to see how such a theoretical set-up, involving as it does the arbitrary assumption of chemical reactions of an unknown nature, can lead to further advances in the elucidation of the mechanism of secretion, and whilst we do not wish to under-estimate the value of such a treatment as a stimulus to further research on the actual nature of these postulated chemical reactions, it would seem that more rapid advances will be achieved by a more direct attack on the nature of these reactions responsible for active transport.

An important contribution to the problem of tubular secretion is the study of Chambers & Kempton (1933) on cultures of the chick embryo mesonephros. It was found by these authors that transplanted fragments of proximal tubules became closed cylinders owing to closure of the ends as a result of the migration and rearrangement of cells. These tubule fragments soon became distended to form cysts, and if the distension was sufficient the cuboidal epithelial cells became flattened. It is quite clear that this distension betokens some secretory activity on the part of the tubule cells: it is, however, not due to a difference of osmotic pressure created by the accumulation of solutes from the incubation medium, since Keosian (1938) has shown that the fluid within the cysts is hypotonic to the outside medium; consequently, the secretion involves the active transfer of water into the lumen against an osmotic gradient, and in this connection it is worth noting that Bieter (1931) has shown that the aglomerular fish can secrete fluid into its tubules against a pressure greater than that existing in the aorta of this animal.

Injection of phenol red into the incubation medium caused the

walls of the tubules (i.e. the epithelial cells) to become coloured, and soon after, colour appeared in the tubular lumen. The succession of changes is shown in Table LXVII and it is evident that the dye changes from the pink colour it has in the slightly alkaline culture medium to a yellow in the cells, indicating that the latter become acid; in the lumen, however, the colour was pink or red, indicating an alkaline medium. In general it was noted that the really great accumulation of the dye occurred in the lumen (if the medium contained 25 mg. $\%$, the lumen might contain as much as 500–700 mg. $\%$, so that it is quite possible to observe a coloration of the lumen with no intermediate coloration of the epithelial cells, and consequently we cannot regard tubular secretion as a concentration of the dye in the cells from which it diffuses passively into the lumen.

TABLE LXVII. THE COLOUR SEQUENCE OF THE WALLS AND LUMEN OF CULTURED CHICK PROXIMAL TUBULES, IN THE PRESENCE OF 100 TO 150 MG. PER LITRE OF PHENOL RED IN THE CULTURE MEDIUM (CHAMBERS & KEMPTON, 1933)

Period of culture	Colour	
	In wall	In lumen
2 min.	None	None
5 min.	Extremely pale pink	None
15 min.	Pale orange	Some pink
35 min.	Orange	Pink
$2\frac{1}{2}$ hr.	Orange	Pink
$7\frac{1}{2}$ hr.	Yellow	Pink
24 hr.	Pale yellow	Red
36 hr.	Colourless	Pink

It was observed that, in contrast to the behaviour of the proximal tubules, distal tubules never became distended, nor did they accumulate dye in their cells or lumen. In cultures in which proximal and distal tubules could be seen to be connected, it was found that the coloration in a phenol-red medium stopped sharply as one proceeded to the distal tubule, and often fluid could be seen to be expelled from the proximal into the distal tubule. If the transplants were maintained at room temperature instead of 39° C., it was found that only the cells were coloured; on raising the temperature to 39° C., the colour appeared in the lumen. If the temperature was reduced to 5° C. or so, no coloration of the tubule lumen or cells was observed; similarly, if a distended cyst containing a high concentration of phenol red

was cooled, the dye returned to the surrounding medium; on re-warming accumulation occurred. From this it was concluded that the uni-directional transfer of dye from medium to tubular lumen requires a high degree of metabolic activity.

With a transplant of a four-day-old mesonephros no coloration of the lumen or cells was observed: preparations more developed than this always showed accumulation, so that we must view the accumulative activity as the result of a differentiation of the more primitive epithelial cells. An interesting fact is that the isolated epithelial cells in the culture, i.e. those not in cysts, showed no evidence of coloration, from which Chambers & Kempton conclude that the cells must be oriented in a definite arrangement in order that they may function as secretory cells in this respect at least (see also Chambers, 1940).

In later papers (Chambers *et al.* 1935; Beck & Chambers, 1935) the inhibition of phenol-red secretion in transplanted tubules by chemical reagents, viz. HCN, H_2S , urethanes and iodoacetate, has been studied in detail. All these metabolic poisons in appropriate concentrations could reversibly inhibit the accumulation and retention of phenol red within the tubular cysts. The concentrations of cyanide for reversible effects were 0.005–0.0005 *M*; of H_2S 0.002–0.001 *M*. The effect of iodoacetate was much less reversible, since after exposure for quite short times degenerative changes were observed in the tubules. Thus in 0.002 and 0.01 *M* iodoacetate degenerative changes occurred within 50 min. At 0.0001 *M* no changes of this nature occurred within 2 hours, but it was found that the retarding action on phenol red accumulation was only slight. Lactate and succinate counteracted to some extent the inhibiting and degenerative effects of iodoacetate, indicating, according to these authors, that iodoacetate inhibits accumulation as a result of the specific poisoning of glycolysis.

Experiments involving Dual Perfusion in the Frog. Mention has already been made of the apparent dual circulation in the kidney of the frog and lower vertebrates whereby the proximal tubules receive their blood supply from the renal portal vein whilst the glomeruli receive theirs from the renal branch of the aorta. That use could be made of this anatomical distinction in studying glomerular and tubular activities separately was suggested by Nussbaum's experiment (1879) involving ligation of the renal artery; later Cullis (1906) perfused the renal portal system directly

and Atkinson *et al.* (1921) suggested a double perfusion, the arterial system being perfused at 24 cm. H_2O pressure and the renal portal at 10–12 cm. H_2O . It has been argued, however, that because of certain anatomical studies which show that the glomerular and tubular circulations are not absolutely separate, the demonstration of the concentration of a substance in the urine above that in the fluid perfused through the portal vein is not satisfactory evidence of secretory activity on the part of the tubule (*vide* e.g. Shannon, 1939; Smith, 1937). Presumably because of this reasoning, the work of Höber and his school has been largely ignored in recent reviews devoted to kidney function. The present writers believe, after a careful study of the experimental results obtained by this technique, that it would be unwise to ignore the many interesting results which have emerged as a result of its use. One must, of course, admit the anatomical possibilities of interconnections between the aortic and portal systems, but as Marshall (1934) points out, this does not rule out the possibility of a functional separation; thus the striking separation of mixtures of dyes effected by the renal tubule when the latter is perfused via the renal portal vein (e.g. a violet mixture of the blue Eriocyanin A and the pink Azofuchsin I gives a pink urine) is difficult to explain unless we assume a functional separation of the two systems. It is true, as we have pointed out earlier in this chapter, that the kidneys perfused in this way rapidly lose their secretory activity, and it is also true that no attempt has been made to allow for the concentration of the substances studied due to a re-absorption of water, and consequently as a quantitative method the technique is bad; but for demonstrating whether a substance is secreted or not it would seem to be quite suitable when large urine/plasma concentration ratios are obtained. It is difficult to believe that a dying tissue will show an accumulatory activity which it would not show in its healthy state, so that the demonstration of this activity in the perfused kidney is strong presumptive evidence that it occurs in the intact animal.

Scheminsky (1929), working in Höber's laboratory, showed that on portal perfusion, the frog tubule concentrates phenol red, whereas cyanol (this is only eliminated by glomerular activity) did not appear in the urine; he also observed that the concentration of the dye could be varied 100-fold in the perfusion fluid

without influencing the rate of secretion, a point which has already been referred to as a result of other techniques. Following up this work Liang (1929) showed that there was apparently a dependence on lipid solubility in so far as the appearance of a dye stuff in the urine on portal perfusion was concerned, and Orzechowski (1930) showed that the degree of concentration of the dye was inversely proportional to its lipid solubility, so that if a lipid insoluble dye, e.g. phenol red, was found to be secreted by the tubules it

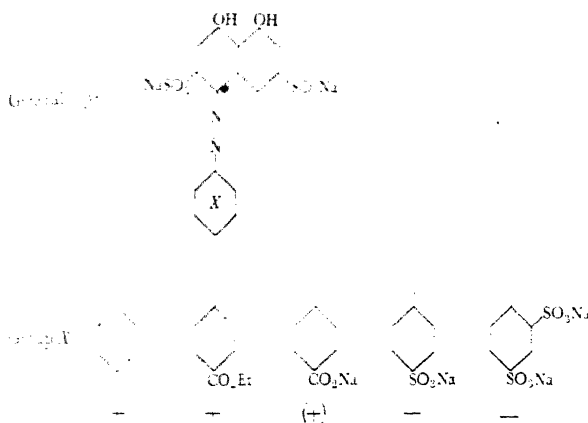


FIG. 67. Secretion of benzene azo-naphthalene derivatives. Above is the general skeleton and below are various forms of the benzenoid ring *X*. Secretion occurs with derivatives marked +, and not with those marked -.

was concentrated to a much greater extent than a lipid soluble one. Höber & Woolley (1940*a*) began their investigations on the nature of the molecule which determines whether it will be secreted or not by developing Orzechowski's observations on the benzene azo-naphthalene dyestuffs; they drew attention to the fact that of five compounds studied by this author the two which were definitely secreted had a polar and non-polar end, whereas of the two which were definitely not secreted no such differentiation of the ends of the molecules pertained (Fig. 67). Experiments with ten benzene azo-naphthalene and ten naphthalene azo-naphthalene dyestuffs gave results in conformity with this general picture; similarly, with more elongated molecules, such as the

dis-azo dyestuffs, it was found that when polar groups were on both ends of the molecule no secretion was observable, whereas if the polar groups of the molecule were confined to one end secretion occurred. As an example Cloth Red 2R (Fig. 68 (a)) was actively secreted, whereas Buffalo Black 10B (Fig. 68 (b)) was not. With the triphenyl methane dyestuffs, an example of which is Cyanol Extra (Fig. 68 (c)), it was found that only one compound was secreted; however, in a later paper Höber & Woolley (1940*b*)

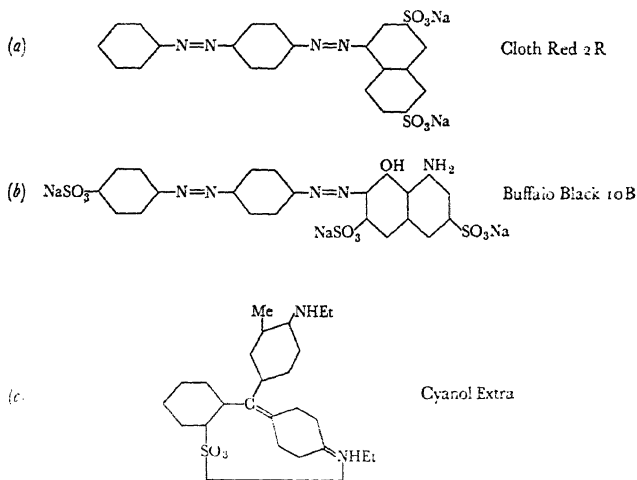


FIG. 68. Structure of certain dyes.

showed that the freshly excised kidney placed in solutions of these dyes will concentrate a number of them (those containing an amino group), whereas if the kidney is first perfused with Ringer solution no secretion of the dyes is observed. For a more complete description and discussion of this work in relation to similar studies with the liver the reader is referred to Höber (1940); if these striking facts can be substantiated by more accurate work on the mammal, Höber's suggestion that the initial step in the secretory process is the adsorption of the secreted molecule on the surface of the cell will be a definite contribution to our knowledge of the fundamental mechanism of this process.

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CHAPTER XXI

THEORIES OF CELL PERMEABILITY

By J. F. DANIELLI

WITH the exception of the theory of membrane potentials, discussed in Chapter xv, we have only touched upon the theory of permeability sufficiently to enable experimental results to be presented in a co-ordinated manner. In this chapter we shall present a general survey of the theory of permeability in qualitative terms. No single theory has yet reached a stage in which it can deal with both non-electrolytes and electrolytes in a quantitative way: we cannot therefore expect to deal quantitatively with the penetration of all diffusible bodies. Lacking this ability, we have restricted our discussion of experimental examples to [*a*] cases where we have confidence that an experiment supports a particular theory; [*b*] cases where an experiment definitely invalidates a theory. The rather large volume of observations falling into neither category we have preferred not to discuss, as little is to be gained thereby.

PROBLEMS OF MEMBRANE STRUCTURE

In Chapters vi and vii we have given the outlines of the various main theories which have been proposed concerning the structure of the cell-plasma membrane. These are (1) that the cell membrane is a thin layer of fatty material; this view was developed by Overton; (2) that the cell membrane is a sieve with pores of molecular dimensions; a theory largely developed by Ruhland, Michaelis and Collander. A theory which is to some extent a compromise between these two extreme views is (3) the mosaic theory of Nathanson, to which Höber has drawn particular attention, according to which the membrane consists of a mosaic made up of areas of more than molecular dimensions, each of different chemical properties. A second modification is (4) the lipid-sieve hypothesis of Collander, according to which the cell membrane consists of a sieve structure superimposed on a lipid layer. Finally (5) we have the suggestion of Gorter & Grendel

that the membrane is a bimolecular leaflet of fatty molecules, and the paucimolecular* layer theory proposed by Danielli & Davson, and Danielli & Harvey, according to which the lipid layer is a few fatty molecules thick, with an adsorbed protein layer at each fat-water interface.

Of these various theories (4) and (5) are modifications and amplifications of (1) and (2) in terms of molecular orientations. When we discussed the experimental results we found one case, that of *Beggiatoa mirabilis*, to which it is possible that theory (2) may be applied in its simple form. Even in this case there are some difficulties in accepting the pore theory (see p. 102). On the other hand, there are a wide variety of cells, of about fifteen different species, which have been shown to have membranes based on a lipid layer. This evidence was based on permeability to non-electrolytes. The study of weak electrolytes and dyes also showed that with these cells, such as *Arbacia* eggs and *Valonia* cells, neutral molecules pass readily, ionised molecules with great difficulty, irrespective of molecular size. This result again is understandable in terms of a lipid layer, but not in terms of a simple sieve theory. In Chapters XII, XIII and XV, dealing with the ions of strong electrolytes, we also found that true permeability to ions is extremely small (with occasional exceptions), in agreement with the lipid layer theory, and that the electrical properties of the membranes of these cells, in so far as they can be interpreted, are also indicative of a lipid layer controlling diffusion into and out of the cell. The paucimolecular layer theory thus rests on a very large body of evidence derived from permeability studies, and on a considerable volume of evidence from other sources, given in Chapter VI.

But this conclusion, that the plasma membrane of most cells has the structure postulated by the paucimolecular layer theory, applies only to the gross, average, structure of the membrane. The evidence available proves that it is true, to a first approximation, and the mass of the evidence available is not capable of proving more than this. So that, while we can accept the paucimolecular layer theory as correct, it is only as a first approximation. Of the details of the structure of the plasma membrane we still know extremely little. In the case of rat,

* Paucimolecular: a term proposed by E.N. Harvey to describe a layer a few molecules thick.

human and mouse red cells the calculations of Danielli suggest that a small fractional area of the cell, not more than 3% of the total, is specially capable of allowing the passage of glycerol. Similar calculations applied to cat red cells would indicate that a very small fraction of the membrane area, less than $1/10^3$, is permeable to Na^+ and K^+ , *in vitro*. We must expect to find similar minor inhomogeneities in the membranes of most cells, as methods suitable for their detection are evolved. These "minor" inhomogeneities are probably only minor when regarded from the point of view of the fraction of the total cell-surface area they occupy: from the point of view of the life of the cell they may well be of major importance.

PROBLEMS OF DIFFUSION KINETICS

There are two major problems of diffusion kinetics. One of these lies in the experimental field, and deals with the problem of calculating from experimental data the rate of penetration of a substance through a membrane in absolute units. This has recently been reviewed by Jacobs (1935), and some of the problems involved have been discussed in Chapters III and IV of this book. The second problem is the theoretical one: What are the details of the process by which a molecule passes through the cell membrane? The key to this problem lies in the study of temperature coefficients.

Q_{10} values of the order of 3 or more are not uncommon, and it was at one time thought that these high Q_{10} values indicated that specific chemical reactions were responsible for the transport of many types of molecule across the cell membrane. Many workers regarded this view with suspicion. M.H. Jacobs, for example, thought it improbable that the cell membrane could provide specific reactions for every type of molecule which organic chemistry can synthesise! Finally Danielli & Davson (1934) pointed out that any type of potential energy barrier was capable of giving rise to high Q_{10} values, and that it was a simple consequence of diffusion kinetics that a slowly penetrating molecule should have a high Q_{10} , and a rapidly penetrating molecule a low Q_{10} . Jacobs *et al.* (1935) made an extensive study of the Q_{10} values of non-electrolyte penetration for eleven species of erythrocytes between 0° and 50°C. , and concluded that their

observations were, "on the whole, in good agreement with the physical theory of the effect of temperature on permeability proposed by Danielli and Davson". There were a number of individual exceptions to the postulated negative correlation between rate of penetration and Q_{10} which both demanded and facilitated a closer analysis of the mechanism of penetration. The mathematical aspects of such an analysis are given in Appendix A of this book.

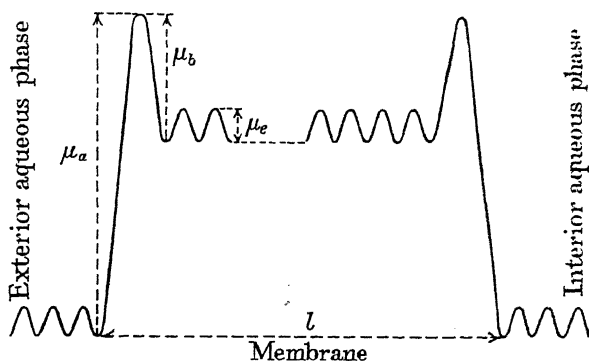


FIG. 69. Potential energy barrier representing the cell membrane.

The potential energy barriers to free diffusion presented by a membrane may be separated into a number of individual potential energy jumps, one of height μ_a met on passing from e.g. an aqueous phase into the interior of a membrane, one of height μ_b met on leaving the membrane, and a number of height μ_e met in the interior of the membrane (Fig. 69). A molecule cannot diffuse over one of these barriers unless it has kinetic energy greater than the μ value concerned. In the simplest case, a homogeneous symmetrical membrane, according to Danielli the permeability is given by

$$P = \frac{ae}{n} = \frac{a \cdot e/n}{\dots\dots(26)*}$$

where a , b and e are rates of diffusion across the potential energy jumps μ_a , μ_b , μ_e and n is the average number of potential energy

* An alternative equation, based on the assumption that Fick's equation may be used for diffusion through the interior of the membrane, is given in Appendix A.

jumps met by a molecule in passing straight across the membrane. This fundamental equation should apply to all diffusing bodies, both electrolytes and non-electrolytes. The more complex cases, where different values of μ_r are met at various points in the interior of the membrane, and where the membrane is non-symmetrical, will give rise to slightly more complex equations of the same type. Where the membrane is a mosaic or pore structure each different individual area must be treated separately, i.e. so

as to obtain a term $\frac{a_r e_r}{b_r + 2e_r}$ for the r th element of area, and then summed: i.e.

$$P = \sum \frac{ae}{b + 2e} \quad \dots\dots (34)$$

In practice the experimental difficulties are such that the main value of this equation at present is to distinguish between homogeneous and non-homogeneous membranes, and between lipid and non-lipid membranes, using the quantitative tests given in Chapter VI. Even in these cases some caution must be observed, owing to the semi-empirical nature of equation (53).

In the simple case of a thin lipid layer the details of the mechanism of penetration, involving the overcoming of hydrogen bond and Van der Waals' forces, have been given in Chapter V, in so far as the details of penetration can be dealt with by physical science to-day. Let us consider several examples. If we take a series of molecules such as methyl alcohol, CH_3OH ; ethylene glycol, $\text{CH}_2\text{OH}.\text{CH}_2\text{OH}$; glycerol, $\text{CH}_2\text{OH}.\text{CHOH}.\text{CH}_2\text{OH}$; erythritol, $\text{CH}_2\text{OH}.\text{CHOH}.\text{CH}_2\text{OH}$; mannitol, $\text{CH}_2\text{OH}(\text{CHOH})_4\text{CH}_2\text{OH}$; we know that each OH group may form hydrogen bonds with water, and that each bond formed will increase μ_a , and therefore decrease a in equation (26). Hence the more OH groups per molecule, the more slowly should the substance pass into the oil layer, and the less will be its permeability. As we have seen in Chapter VIII, this is just the type of behaviour which is observed. Secondly, since each OH group increases μ_a , we see from equation (62) of Appendix A that the Q_{10} value should increase with each additional OH group added to a molecule. This also is the type of behaviour observed experimentally. But if we take a series $\text{CH}_3.\text{OH}$, $\text{CH}_3.\text{CH}_2\text{OH}$, $\text{CH}_3.(\text{CH}_2)_2\text{OH}$, $\text{CH}_3.(\text{CH}_2)_3\text{OH}$, etc., where the number of CH_2 groups is varied but the number of OH groups is kept constant, the values of μ_a , μ_b , μ_e will change

quite slowly, since no hydrogen bond forces are changed in passing from molecule to molecule; only the Van der Waals' forces are changing. Consequently, we predict that for such a series the Q_{10} values will vary comparatively slowly with increase in molecular weight. This again is what is observed experimentally.

The mechanism of diffusion through the more specialised areas of the cell membrane, which are specially adapted for penetration by specific molecules, cannot be profitably discussed until much more information is available.

The Variation of P and Q_{10} with Temperature. In Appendix A it is shown that for diffusion through a thin homogeneous lipid layer whose structure is not significantly varying with temperature, the permeability varies exponentially with temperature, and if $\log P/\sqrt{T}$ is plotted against $1/T$ a straight line should be found, subject to certain assumptions. Fig. 70 gives an example of this, calculated from the results of Jacobs *et al.* (1935).^{††} Most of the accurate work on the temperature variation of P obeys this relationship, and failure to obey it means that probably a complicating factor, such as inhomogeneity or marked variation in structure with temperature, is present.

As shown in Appendix A there is theoretically a negative correlation between $PM^{\frac{1}{2}}$ and Q_{10} for both slowly and rapidly penetrating molecules. Failure to obey this also means a complicating factor is present. The Q_{10} itself should also decrease with increasing temperature, and in fact usually does, though there are a number of individual exceptions to this rule.

The Penetration of Water. Water is a rapidly penetrating substance, and its rate of penetration will in most cases be given by equation (27), $P = a/b \cdot e/n$. The rate of penetration is thus determined by the partition coefficient a/b , the viscosity, proportional to e , and the thickness, proportional to n . For a membrane having the same viscosity as water, the permeability to water will be less than that of an equal thickness of water if the partition coefficient is less. The interior of the cell membrane is mainly a hydrocarbon liquid or solid composed of CH_2 chains and sterol residues, etc. and in such a system the partition coefficient of water will be of the order of 10^{-3} or less. Due then to the partition coefficient, the permeability to water will be at least 10^3 times less than that of an equal thickness of water. Then the viscosity term e for such a liquid is probably between 10^{-2} and 10^{-5} of

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that of water. This reduces the permeability by a factor of the order of 10^4 – 10^5 , giving a total reduction of 10^6 or more.²² It is kable that such a large effect can be produced by a mobile

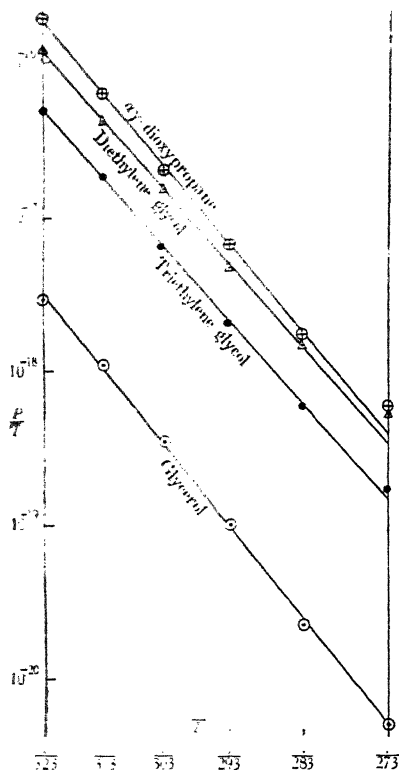


FIG. 70. Effect of temperature on permeability. $\log P/\sqrt{T}$ plotted against $1/T$, for ox red cells.

membrane only 10^{-6} cm. in thickness. Experimentally, it is found that the permeability to water is very small, smaller than the maximum value just predicted.

The Role of Partition Coefficients. The relationship between permeability and partition coefficients has been the subject of speculation and enquiry ever since the first studies of permeability were made. In Appendix A formal relationships for two groups

of molecules are given, for certain slowly penetrating molecules (equation (28)) and for certain rapidly penetrating molecules (equation (27)). Examples of the application of these are given in Chapter VIII. It remains to discuss the physical basis of these relationships.

In the case of certain types of rapidly penetrating molecules equation (26) reduces to $P = a/b \cdot e/n$. Since the oil-water partition coefficient is a/b , we can see that if e/n were constant, the permeability of a homogeneous lipoid layer would be directly proportional to the partition coefficient. The term e/n is subject to variations, which, whilst for any single membrane they are probably small compared with the variations in a/b , are still large enough to give rise to minor differences between the series of molecules arranged in order of partition coefficients and permeability constants.

In the case of slowly penetrating molecules $P = a/2$, and the partition coefficient is a/b . If we divide both sides of this equation by b , we obtain $P/b = 1/2 \cdot a/b$. In other words, if b were constant, the rate of penetration of slowly penetrating molecules would also be proportional to the partition coefficient. Actually, b does vary, but the variation is often small compared with the variation in a/b . The reason for this is that the chief variable affecting a is the hydrogen-bond force between polar groups and water, involving an energy of the order of 5000 calories per group, whereas the chief variable affecting b is the activation energy of diffusion of non-polar groups into water, involving an energy of the order of 2500 calories per group. As the former force varies within much wider limits than the latter force, it follows that a varies within much wider limits than b . Hence we again predict that molecules arranged in the series of partition coefficients and of permeability constants will be in *roughly* the same order, but there will be many differences in detail due to the other variables involved. This predicted rough parallelism with minor variations between the two series is what is in practice found, both for slowly and for rapidly penetrating molecules.

It is quite clear that if we compare the permeability of two membranes which differ in their partition coefficients, other things being equal, they will have different permeabilities to the same molecule. The partition coefficients of substances like urea, glycerol and sugars in acidic, neutral and basic oils are quite

different. Table LXVIII shows the relative coefficients of some typical molecules in olive oil, which is slightly acidic, and in olive oil + 20% oleic acid, which is strongly acidic; these results of Collander's give a rough idea of the order of magnitude of the possible variation due to differences in partition coefficients. Höber and Collander* have particularly emphasised the importance of differences in partition coefficients, both in different membranes, and in different areas of the same membrane. Recently Schulman and Rideal, in a series of papers, have shown

TABLE LXVIII

The figures give the ratio: partition coefficient in olive oil + 20% oleic acid : partition coefficient in olive oil

Triethyl citrate	1.0
Glycerin monomethyl ether	1.5
Ethyl alcohol	1.7
Propylene glycol	1.7
Erythritol	2.0
Glycerol	2.1
Ethylene glycol	3.3
Formamide	6.4
Propionamide	10
Acetamide	11
Ethyl urea	20
Urea	35
Urotropin	86

that even in monolayers of fatty substances and proteins, complexes are very readily formed due to the interaction of two different molecules, or to the interaction of similar molecules containing a variety of chemical groups. These interactions may involve 1. Van der Waals' forces, 2. electrostatic dipole-dipole interaction, 3. dipole-ion interaction, 4. ion-ion interaction. These forces are, of course, the same as those involved in giving rise to partition coefficients, and consequently these observations of Schulman and Rideal are of great importance in showing experimentally that differences in partition coefficients extend even to monolayers, and in fact are sometimes more marked in monolayers than in bulk phases. In so far as these interactions influence permeability, they do so by changing the partition coefficient and effective viscosity in the case of rapidly penetrating molecules (obeying the equation $P = a/b \cdot e/n$). In the case of slowly penetrating molecules, obeying the equation $P = a/2$, the

* Some examples quoted from Collander are given in Chapter VIII.

effective resistance to penetration lies exclusively at the membrane interface, and these interactions affect permeability (1) by changing the activation energy μ_a , and (2) by causing an increased adsorption at the oil-water interface—the Traube effect (see below). These experiments of Schulman and Rideal thus fully substantiate the importance of variation in partition coefficients which was emphasised by Höber and Collander. We may thus safely state that there is a range of at least 10^2 – 10^3 in the partition coefficients of the same molecule in different lipid membranes, and consequently a possible difference of this order in permeability of different cells to the same molecular species.

The Traube Effect. Traube suggested that, since adsorption at an oil-water interface increases the effective concentration of the adsorbed compound at the interface, surface-active compounds will for this reason penetrate more rapidly than their less surface-active homologues. This principle of Traube's is not valid in the case of rapidly penetrating molecules obeying the equation $P = a/b \cdot e/n$, since the concentration in the membrane interior is that given by the partition coefficient, and is in equilibrium with the concentration in the bulk aqueous phase, so that the concentration in the surface phase is of no importance. But for molecules penetrating slowly and obeying the equation $P = a/2$, the rate of penetration is affected by the concentration in the surface phase: this is because the point constituting the effective and dominant barrier to penetration is the oil-water interface only: the condition of the interior of the membrane is of no consequence to a molecule obeying this equation.

The ratio—concentration in surface phase : concentration in bulk phase—is given roughly by

$$\frac{C_s}{C} = e^{\frac{750x}{RT}}, \quad \dots\dots(35)$$

where x = number of CH_2 groups per molecule.* These adsorbed molecules will be oriented, often in a way favouring penetration into the interior of the lipid layer, so that the rate of penetration may be increased by adsorption, due to the favouring steric factor. This phenomenon may be modified by the formation of the complexes described by Schulman and Rideal, which will give

* $750x$ is the difference in potential energy in calories, between the surface and bulk phases, for a compound containing x non-polar groups.

rise to a possible difference in permeability of different membranes to the same molecule of the order of 10^2 – 10^3 , in the case of molecules obeying the equation $P=a \cdot 2$.††

Thus we see that as a general theory of permeability Traube's suggestion is invalid, the close correlation between permeability rate and surface activity being due largely to the fact that with the substances chosen the surface activity ran parallel with the oil-water partition coefficients.

The Influence of Molecular Volume. The permeability of non-lipoid porous membranes changes sharply when molecular diameter exceeds that of the average pore size of the membrane, so that molecular volume is a good guide to the permeability of such membranes. Collander & Bärlund (1933) and Collander (1937) have attempted to apply this concept to lipid membranes also; by plotting permeability against partition coefficient for a large number of substances penetrating the same membrane, a distribution of points was found such that if molecules of a given partition coefficient are compared, it is found that those having small molecular volumes penetrate much more rapidly than those with large molecular volumes. An example of this is given on p. 97. Collander concluded from such results that the cell membrane distinguishes between molecules in virtue of variation in their molecular volumes.

This method of plotting was empirical, and not based on qualitative considerations. From the theory of permeability of thin lipid layers it has been shown (Appendix A) that, under certain conditions, for a homogeneous membrane $P \cdot M^{\frac{1}{2}}$ should be plotted against partition coefficient in the case of rapidly penetrating molecules, and $P \cdot M^{\frac{1}{2}} e^{\mu_2/RT}$ against partition coefficient in the case of slowly penetrating molecules. When such plots are made of Collander's results linear relationships are found, in accordance with theory (see e.g. p. 99) and there is no selective action exerted by molecular volume. Hence Collander's conclusion was probably unjustified; the distribution of points he found is semi-fortuitous, and due to the fact that $M^{\frac{1}{2}}$ and $M^{\frac{1}{2}} e^{\mu_2/RT}$ tend to run parallel to molecular volume.

The experimental data available show that for most cells molecular volume probably plays a very minor role, if any. An exception to this general rule is *Beggiatoa* (p. 102), in which the sieve effect may be very pronounced.

The Influence of Membrane Viscosity. In dealing with permeability to water we have pointed out that rapidly penetrating molecules, obeying the equation $P = a/b \cdot e/n$, find part of the resistance to free penetration in diffusing through the interior of the plasma membrane. Consequently, when comparing the penetration of different membranes by the same substance, the effective* membrane viscosity is an important variable. The viscous resistance term e may easily vary by a factor of 10^3 for different membranes. We have also already found that the partition coefficient of the same compound in different membranes may vary by at least 10^2 – 10^3 . This gives us a total theoretical variation in the permeability of different cell membranes to the same compound of at least 10^4 – 10^5 -fold. To this must be added a further factor of about tenfold, due to variation in n , i.e. in the thickness of the membrane. This is quite sufficient to account for the variations found in Table XXII, p. 108.††

Slowly penetrating molecules, obeying the equation $P = a/2$, have a permeability which is independent of the interior of the membrane, and therefore are not affected by the membrane viscosity. Species variations for such molecules probably arise largely from complex formation at the membrane interface. In such cases it is also possible that some at present undetected physico-chemical factor is involved. Complex formation alone, however, may give a variation of 10^2 – 10^3 in permeability to the same molecule.

The "Active Patch" Theory of Membrane Specificity. There is a considerable volume of evidence that small fractions of the erythrocyte cell membrane are specifically permeable to certain compounds. A fraction of 3% or less is specifically permeable to glycerol in human, rat and rabbit cells. When cat erythrocytes are placed in isotonic KCl, a fraction of less than 0.1% is permeable to Na^+ and K^+ . When rabbit cells are subjected to sub-haemolytic swelling, a fraction of less than 1% is permeable to K^+ . The evidence for the existence of this local differentiation of the cell surface is possibly supported by the action of certain lysins, which appear to attack only a restricted fraction of the membrane surface (Ponder). In the case of the ion-permeable areas it is possible that the K^+ ions leak out through simple pores or cracks

* The effective viscosity is not necessarily identical with the viscosity measured by a macroscopic method.

in the membrane, but the specific permeability to glycerol involves a specially differentiated area which has not the same high permeability to other molecules of the same molecular diameter. Furthermore, the specific permeability is eliminated by poisons such as acid and Cu^{++} (Jacobs, Glassman & Parpart). For example, rabbit cells normally have a high permeability to glycerol and a Q_{10} for glycerol penetration of about 1.2, but in the presence of CO_2 or traces of Cu^{++} the permeability is greatly reduced, to about the same level as for ox cells, and the Q_{10} raised to between 3 and 4. Thus in many ways these specifically permeable areas resemble the 'active patches' of a catalyst.

It is difficult to obtain information about the chemical nature of such patches. Direct chemical analysis will probably be quite useless. Dziemian (1939), in a recent most interesting paper, has shown that there is no correlation between the lipid composition and membrane permeability of red cells, even when the non-specific permeability is considered, so it is hardly to be expected that chemical analysis will be useful in the much more delicate problem of specific permeability. A certain amount of information can be gained in other ways. For example, the Q_{10} of 1.2 involves an activation energy of only a few thousand calories, corresponding to the passage of at most one OH group into a fatty layer, whereas in fact three groups must penetrate, involving a Q_{10} of at least 2.3 at 20°C . Hence it is improbable that the areas concerned contain much lipid. But they must contain groups permitting diffusion of glycerol by breaking only one hydrogen bond at a time, for example, CO_2^- groups and OH groups. The inhibitory effect of acidity and Cu^{++} also suggests that membrane anions are involved, and the observation of Wilbrandt (1939*b*) that interference with the phosphorylation processes of red cells affects permeability suggests that PO_4^- groups may be involved. All these deductions are of course of a very preliminary nature.††

PROBLEMS OF THE ACTION OF IONS

In an abnormal ionic environment cells have abnormal permeability. Recently some quantitative treatments of the action of ions on interfaces have been derived which throw a good deal of light on this problem. Danielli (1936, 1937) pointed out that at the surfaces of most cells there are fixed anions. Hence there will be a Gibbs-Donnan equilibrium existing between the surface

phase and the bulk phase, which will lead to an unequal distribution of ions between these phases. For example, for H^+ and Na^+ we have

$$\frac{[H^+]_s}{[H^+]_b} = \frac{[Na^+]_s}{[Na^+]_b} = \text{constant}, \quad \dots\dots(36)$$

where the suffixes s and b denote surface and bulk concentrations. Most cells have a value of rather less than 10 for this constant when in physiological saline or plasma, i.e. the surface of the cells is more acid than the saline or plasma by 1.0 to 0.3 pH units. From the equation it is clear that if the number of fixed anions is kept constant, $[H^+]_b$ is kept constant, and $[Na^+]_b$ diminished, then owing to the fixed anions in the surface phase $[Na^+]_s$ will fall off much more slowly than $[Na^+]_b$, and consequently $[H^+]_s$ will increase, i.e. dilution of physiological saline with either water or non-electrolyte solution will increase the acidity of the surface phase. Danielli, using the Debye-Hückel theory to calculate the thickness of the surface ionic layer, has recently shown that the effect of dilution in e.g. solutions of $NaCl$ can be calculated quantitatively.

Hartley & Roe (1940) have recently shown that surface pH may also be calculated from cataphoretic velocity measurements, and in some cases measured directly by using surface colorimetric indicators. The values of the surface pH given by all three methods are in reasonable agreement.

Wilbrandt (1939*a*), has shown that the Gibbs-Donnan relationship may be extended to deal with the distribution of sodium and calcium ions between surfaces and bulk phases. Two particularly important applications to physiology may be distinguished: (1) if, when cells are suspended in isotonic non-electrolyte, *either* sodium or calcium will produce a given effect, e.g. reduction in permeability to water, the quantities of sodium and calcium required to produce the same intensity of action, e.g. the same change in permeability, are given by

$$\frac{[Na]_b}{\sqrt{[Ca]_b}} = \text{constant}. \quad \dots\dots(37)$$

(2) If *both* sodium and calcium are present, the relationship between the quantities in the surface and bulk phases is given by

$$\frac{[Na]_s}{[Na]_b} = \frac{\sqrt{[Ca]_s}}{\sqrt{[Ca]_b}} = \text{constant}. \quad \dots\dots(38)$$

Let us take $[\text{Na}]_i = 2.0 M$, $[\text{Na}]_s = 0.1 M$, $[\text{Ca}]_s = 0.002 M$, which are reasonable values for a properly balanced physiological saline. Then we obtain

$$[\text{Ca}]_i = \frac{2^2}{0.1^2} \times 0.002 = 0.8 M,$$

i.e. although the Na : Ca in bulk is 50 : 1, in the surface phase it is 1 : 0.4, i.e. the disproportionate ratio of 50 : 1 in bulk yields a much more balanced ratio in the surface phase. This phenomenon is probably of considerable importance in relation to salt antagonism. The above equation also shows that dilution of a balanced salt solution with water decreases the Na : Ca ratio in the surface phase, i.e. increases the relative amount of Ca in the surface. These relationships have been studied by chemical analysis of monolayers (Webb & Danielli, 1940).

Obviously such distributions of ions will be of importance in relation to the actions of the physiologically active bases, such as histamine, adrenaline, acetyl choline, curare, the purine bases, etc. on cell permeability, but calculation is complicated in such cases by oil solubility, and interpretation must await the study of anionic monolayers spread on solutions of these ions.

The Action of Low Concentrations of Ions. The thickness d of the ionic layer composing the ionic surface phase of a cell is given by the theory of Debye and Hückel as

$$d = \frac{3.1 \times 10^{-8}}{\sqrt{[\text{Na}]_s}} \text{ cm. at } 0^\circ \text{ C.,}$$

for cells in NaCl solution. Thus we can see that if most of the Na^+ is washed away with e.g. sugar solution, the value of d will be greatly increased. The value of d also indicates the degree of separation of the positive charges of the mobile cations and the fixed anions of the surface phase. The mutual repulsive force between any two adjacent pairs is proportional to the distance of separation of the positive and negative ions.

As the distance between any oppositely charged ionic pair is increased by dilution, the mutual repulsive force between two adjacent pairs will increase. As an example of this increased repulsive action we may take the results of Adam & Miller (1933), which show that there is much less lateral adhesion between the molecules of sodium palmitate monolayers when on dilute

solutions than when on concentrated solutions. Thus there will be an increase in the lateral repulsive forces in the cell membrane on washing cells with non-electrolyte (provided the number of fixed anions is unchanged), which will tend to loosen the packing of the molecules constituting the plasma membrane. Such loosening of the structure would increase the cell permeability, especially to substances like water and ethylene glycol which have rather low oil-water partition coefficients but yet obey the equation $P = a/b \cdot e/n$. That this is so, is well established by the work of Jacobs & Parpart (1932) and Wilbrandt (1939*a*, on red cells, and of Lucké & McCutcheon (1929) on *Arbacia* eggs. Thus addition of salts to cells in non-electrolytes has at least three direct effects: (1) increasing the lateral adhesion of membrane molecules, (2) increasing the surface phase pH, (3) decreasing the thickness of the ionic double layer. All of these variables may be to some extent involved in defining cell permeability.

TABLE LXIX. CONCENTRATIONS OF SODIUM AND CALCIUM HAVING THE SAME EFFECT IN REDUCING THE PERMEABILITY OF RED CELLS TO WATER AT CONSTANT pH

$[Ca^{++}]_b$	0.042	0.021	0.010	0.0057	0.0029	0.0014	0.00072	0.00036
$[Na^+]_b$	0.022	0.017	0.010	0.0079	0.0056	0.0040	0.0030	0.0016
$\frac{[Na^+]}{[Ca^{++}]}$	0.11	0.12	0.10	0.11	0.11	0.11	0.12	0.10

TABLE LXX. CONCENTRATIONS OF SODIUM AND CALCIUM HAVING THE SAME EFFECT IN REDUCING THE LEAKAGE OF K^+ FROM RED CELLS IN SUCROSE

	0.00113	0.00074	0.00056	0.00037	0.00024	0.00015
$[Na^+]_b$	0.0161	0.0128	0.0111	0.00926	0.00762	0.00546
	0.48	0.47	0.47	0.48	0.49	0.45

Wilbrandt (1939*a*) has shown, in the case of the erythrocyte, that quantitatively, the same reduction in permeability to water is given by either sodium or calcium in relative concentrations such that $[Na]_b/\sqrt{[Ca]}_b = \text{constant}$. Similar results have been obtained on the leakage of K^+ from red cells suspended in sugar solution (Wilbrandt 1940). These quantitative treatments are given in Tables LXIX and LXX.

From this discussion it will be evident that we have now some of the elements necessary for a clear understanding of the actions of small amounts of salts on cell permeability. But a great deal more work remains to be done, especially in connection with ions which form non-ionic compounds, or weak electrolytes, with the anions fixed on the cell surface.

The Antagonistic Action of Ions. The classical studies of Ringer (1882, 1883, 1886) on the toxic effects of pure isotonic sodium-chloride solutions on the irritability of skeletal muscle and the frog's heart beat—effects which could be antagonised by the addition of small quantities of Ca and K giving a Na : K : Ca ratio of 100 : 2.4 : 1.6 in the case of frog Ringer solutions, and 100 : 1.7 : 1.1 in the case of mammalian Ringer solutions—introduced the concept of the antagonistic action of salts on physiological phenomena. This phenomenon must not be confounded with the action of Na and Ca on the “non-electrolyte effects” in the case of the erythrocyte just described; in these instances Na and Ca both exerted the same effect, i.e. they decreased the abnormally large permeability obtained by placing the cells in an electrolyte-free medium, whereas the phenomena of antagonism are generally exhibited in isotonic salt solutions (e.g. sea water, Ringer solution) and the two antagonistic ions exert, at least when they are both present in solution, opposing effects on the tissue or cell.

In permeability studies the antagonism between Na and Ca has attracted most attention and has been demonstrated very neatly by Osterhout (1911), who showed that plasmolysis of *Spirogyra* cells could be brought about in a mixture of NaCl and CaCl₂ in the molal proportions of 20 : 1, whereas pure solutions of NaCl and CaCl₂ were unable to do so. This author has also studied in great detail the antagonistic action of these and other ions on the conductivity of *Laminaria* fronds (1922). The influence of Ca in inhibiting the increase of permeability normally occurring on placing *Arbacia* eggs in a non-electrolyte medium has already been referred to; in this case Na appears not to be effective, rather does it, along with other univalent cations, cause a further increase in permeability. In mixtures with Ca salts antagonism occurs, i.e. when both are present in appropriate concentrations the resulting permeability is intermediate between the values produced by them singly. Thus a concentration of 0.016 M NaCl completely abolishes

the effect of $0.0005\text{ }M\text{ CaCl}_2$, i.e. the Ca may be said to be thirty times as effective as Na.

Clowes (1916) found that when oils, containing e.g. oleate ions, are shaken with sodium chloride solutions an emulsion is formed of the type oil-in-water, i.e. oil droplets in a continuous phase of water. But with calcium chloride the emulsions are water-in-oil, i.e. the continuous phase is oil. With mixtures of sodium and calcium ions, oil-in-water emulsions are formed when the ratio Na : Ca is greater than about 75 : 1, and water-in-oil emulsions when the ratio Na : Ca is less than about 75 : 1. At the boundary ratio 75 : 1 the type is variable. The ratio 75 : 1 is about the same as that in a balanced saline, and led Clowes to suggest the cell membrane is normally an emulsion of indeterminate type, and that the increase in permeability when the relative amount of sodium is increased is due to conversion into an oil-in-water emulsion, so that there will be many continuous tracts of water passing right through the cell membrane, permitting ready penetration of water-soluble substances. Calcium, on the other hand, might convert the membrane into a water-in-oil emulsion, leaving no continuity between the external and internal aqueous phases, thus reducing the permeability. In the absence of quantitative information about the thickness of the plasma membrane, this was a reasonable and plausible theory.

Between 1925 and 1930 evidence was accumulated by Gorter, Fricke and Cole to show that the thickness of the cell membrane lies between 10^{-6} and 10^{-7} cm. (see Chapter VI). This is a very thin layer, at most only half a dozen fatty molecules thick, probably less. In this space it is difficult to see how phase reversal can take place in a reversible manner. Mainly on this consideration Danielli & Davson (1934) put forward an alternative theory, that the action of the ions was to cause variations in the partition coefficient of the membrane, thereby changing its permeability. Sodium salts of anions such as $-\text{CO}_3^-$, $-\text{PO}_4^{--}$, SO_4^{--} are known to dissolve water more readily than calcium salts of the same anions, so that the larger the proportion of sodium in the membrane, the higher would be its permeability to water. In other words, the reversible changes in permeability were attributed to changes in chemical composition of the membrane which directly affect the membrane partition coefficients.

At the time this second theory left unexplained the significance of the Na : Ca value of 50-100 : 1, at which the action of the two ions is usually balanced. But the calculation given above [p. 324] shows that at this bulk ratio of 50-100 : 1, the ratio Na : Ca in the surface phase, in molal units, is roughly 2 : 1. Thus the importance of the bulk ratio 50-100 : 1 arises from the fact that at the cell surface balance can be achieved only at a molar ratio of the order of 2 : 1 [1 : 1 in equivalents], and, as Wilbrandt has pointed out, the reason why Clowes found the same ratio in his emulsion phase reversal experiments may be that in this case also a surface ratio of Na : Ca = 2 : 1 is required, involving a bulk ratio of about 75 : 1. Similarly, Danielli [1937] found that with saline at pH 5 calcium oleate and calcium palmitate surfaces have a higher interfacial tension than sodium oleate and sodium palmitate surfaces, and in mixtures of the two ions an intermediate tension is found at the "physiological" value of about 75 : 1. The common feature of such phenomena is the requirement of an approximately equal number of equivalents of Na^+ and Ca^{++} at an interface, which is attained by a bulk ratio of about 75 : 1, when using physiological salt concentrations.

Turning now to the *mechanism* whereby Na^+ increases, and Ca^{++} decreases, water solubility in the cell membrane, we find that calcium produces closer packing by at least two factors: (1) increased lateral adhesion due to reduction in thickness of the ionic double layer, as given on p. 324; (2) increased lateral adhesion due to the cross-binding action of the divalent cation on several monovalent membrane anions (Danielli, 1937). Both factors tend to squeeze out of the membrane extraneous non-fatty molecules such as water and to increase the membrane viscosity.

The foregoing theory gives a basis for the explanation of the general phenomenon of Na-Ca antagonism, but that is all that can be hoped for at the present. If it is allowed that each ion is capable of exerting specific effects on the membrane of any cell, which are not determined completely by the net electrical charge of this ion or by its sign, it follows that antagonistic action by many pairs or groups of ions may be expected in permeability phenomena, e.g. Na-K, K-Mg, Ca-Mg and so on, and it further follows that these antagonistic actions are unlikely to fall within the scope of any general theory which does not take into account the more specific relationships between the individual ions and

the components of the membrane. The literature of general physiology is indeed well supplied with such examples of antagonism, many of which are imputed, often on the basis of insufficient evidence, to permeability changes, but the adequate explanation of any single case must depend on a much more complete quantitative investigation than any so far presented. The reader who wishes to follow this particular problem further would be well advised to read Höber's masterly summary of the facts (1924, Chapter ix).

The Penetration, etc. of Weak Acids and Weak Bases. In Chapter xiv we have pointed out that weak acids and weak bases frequently penetrate as the undissociated compounds, though weak bases also frequently penetrate after forming a complex with an acid component of the cell membrane. We must now consider the effect of surface ionisation on penetration of these compounds. With rapidly penetrating compounds obeying the equation $P = a/b \cdot e/n$, surface effects are unimportant, and penetration will be uninfluenced by surface ionisation, except in so far as the structure of the membrane is altered. With slowly penetrating compounds obeying the equation $P = a/2$, the concentration of the penetrating molecules in the interfacial phase is most important. Consider the penetration of a weak acid HA of $pK_a = 5$ which penetrates through, say, the *Arbacia* egg membrane as HA, not as A^- . The surface phase of an *Arbacia* egg in isotonic (0.5 M) saline has about five times the hydrogen-ion concentration of the saline. In 0.5 M NaA at pH 7 the concentration of $[HA]_b$ is

$$[HA]_b = [H^+]_b [A^-]_b \times 10^5 = 10^{-7} \times 0.5 \times 10^5 = 0.05 M.$$

In the surface phase the Donnan equilibrium gives (ignoring surface activity)

$$[H^+]_s [A^-]_s = [H^+]_b [A^-]_b.$$

So that
$$[A^-]_s = \frac{10^{-7} \times 0.5}{5 \times 10^{-7}} = 10^{-1},$$

and

$$[HA]_s = [H^+]_s [A^-]_s \times 10^{-5} = 5 \times 10^{-7} \times 10^{-1} \times 10^5 = 0.005 M.$$

Hence the concentration of the penetrating form HA is the same in the surface as in the bulk phase, and therefore its rate of penetration cannot be affected by surface ionisation.

In the case of the ions, the surface concentration of anions is decreased (e.g. A^- by a factor of 5 in the above calculations) and

of cations is increased by a factor of 5 for H^+ in the previous calculation. These changes, however, are purely due to electrostatic forces, which in these circumstances will also change the activation energy, μ_a , by a factor exactly compensating for the changes in concentration. Hence we find that, unlike the concentration changes produced by surface activity (the Traube effect), the changes in concentrations of anions and cations at the membrane surface due to the ionisation of the surface will not directly affect the permeability of that ion.

A factor which may prove to have importance is the possible change in structure of the membrane, and in its ability to form complexes with solute molecules, when the surface pH is changed. In this connection it would be very interesting to know the changes in surface pH of cells in solutions of weak acids, and of bases such as veratrin, guanidine and acetylcholine. This information can, in some circumstances, be obtained from cataphoretic velocity studies (G.S. Hartley, 1940).

PERMEABILITY TO IONS

Thick Membranes. In Chapter xv we have given the theory of Michaelis, Wilbrandt, Teorell and Meyer & Sievers for the diffusion of ions through porous and fatty membranes of such thickness that diffusion across the membrane-water interface is very much more rapid than diffusion across the interior of the membrane. These theories apply to ions obeying the permeability equation $P = \Sigma a \cdot b \cdot e \cdot n$, and are fairly satisfactory when applied to the potentials developed across a membrane. The practical problem of the rate of transport of any individual ionic species across a membrane still presents many difficulties.

The formulae for membrane potentials due to Teorell (1935), and Meyer & Sievers (1936), would probably be applicable to cell membranes in cases where all the ions obey the equation $P = a \cdot b \cdot e \cdot n$, but not to other cases. The practical problem of finding when, and which, ions obey the equation $P = a/b \cdot e/n$, i.e. cases where the rate of diffusion across the membrane-water interface is relatively rapid, has yet to be solved.

Diffusion Across Thin Membranes. Where the rate of diffusion of an ion across the membrane-water interface is comparable with, or much less than, the rate of diffusion across the interior of a

membrane, the equations $P = \Sigma ae (nb + 2e)$ and $P = \Sigma a^2$ will be obeyed respectively. No theory has yet been developed for the potentials in such cases. We are thus restricted to discussion of the factors which may influence the terms a , b , e and n . The terms a , b and e will each involve an activation energy, μ_a , μ_b , μ_e .

n is proportional to the membrane thickness, and will vary as the thickness varies. e is given by $n\lambda (n+1) D$, where D is the Fick diffusion constant and λ the average distance between two adjacent potential energy minima in the interior of the membrane (see Appendix A). Thus anything affecting the Fick diffusion constant of an ion in the interior of the membrane will affect its rate of penetration. Electrostatic potentials set up by the diffusion of ions or by fixed charges in the interior of the membrane, and in the case of porous membranes, changes in pore size or ion diameter, will also affect the rate of diffusion.

The term a is the constant defining diffusion across the membrane interface in the direction water \rightarrow membrane. The activation energy μ_a involved in a is the minimum kinetic energy necessary for carrying an ion from the aqueous phase across the interface. This involves (1) sufficient energy to break loose from the aqueous phase, (2) sufficient energy to make a "hole" in the membrane large enough to contain the penetrating ion. (2) will often be small in biological systems, but breaking loose from the aqueous phase may involve a large kinetic energy. For example, penetration of an ion into a lipoid membrane, such as the cell membrane, will involve either (a) tearing the ion completely away from the coat of water molecules which are normally bound tightly to it by electrostatic forces, or (b) breaking the hydrogen bonds between this bound water and adjacent water molecules, so as to remove the ion complete with its coat of water, or, of course, some step intermediate between these two. Other factors influencing μ_a will be the diffusion potentials, and the electrostatic potential barrier at the membrane surface due to the fixed ions and oriented dipoles of the membrane molecules.

The term b defines the rate of diffusion across the interface in the direction membrane \rightarrow water. The activation energy μ_b involved in b is composed, for lipoid membranes, of terms for the Van der Waals' forces to be overcome, for the membrane interface electrostatic potential, for the diffusion potential, and for the energy necessary to make a hole in the water.

If a , b and e can be expressed in terms of the same equation as that found useful for non-electrolytes, e.g.

$$a = r\phi_a \cdot \frac{e^{-\frac{\mu_a}{RT}}}{\sum_{i=1}^n e^{-\frac{\mu_i}{RT}}} \quad \text{see Appendix A, 53,}$$

where r is a constant, and ϕ_a is the probability that a molecule or ion will diffuse if possessed of kinetic energy equal to μ_a , several more factors are given. E.g. the orientation of the ion is important, being involved in ϕ_a , ϕ_b and ϕ_e . Also the mass is involved. In aqueous solution the rates of diffusion of ions are believed to be more nearly proportional to their radii than to their masses, so the validity of equation (53) for ions may well be doubted. If a term for the ionic mass is not involved, then one for the ionic radius may be. It is quite possible, however, that through media of viscosity much greater than water, such as the cell membrane, equation (53) is roughly true for ions also. Cowan, for example, found that the action of ions on the excitability of crab's nerve is roughly correlated with the ionic mass with the small ions K^+ , Rb^+ and Cs^+ .

Lastly, the Traube effect may also affect a , but will only be of importance for cases where $P = \Sigma a^2$.

Summing up, the permeability to an ion is defined by three sets of factors:

- (1) interface factors a and b ,
- (2) membrane "viscosity" factor e ,
- (3) thickness factor n .

These factors are themselves influenced by

- i) mass or radius of ion (lipoid membranes); radius of ion and pore radius (porous membranes),
- ii) orientation factors ϕ_a , ϕ_b , ϕ_e ,
- iii) adsorption (Traube effect),
- iv) values of μ_a , μ_b , μ_e .

And these terms involve effects due to

- (α) diffusion potentials,
- (β) chemical nature of membrane,
- (γ) chemical nature of diffusing ion,
- (δ) charge on membrane surface, and on membrane pores.

With regard to the formulae available, equation (26),

is certainly valid; Turton's alternative equation (44) (Appendix A) may be more convenient for thick membranes, but cannot be accurate for thin ones. The expression of a , b , e for ions in the terms $r\phi_{\infty} \sqrt{\frac{kT}{5\pi\lambda\epsilon}} e^{-\frac{\mu}{RT}}$ is a possible relationship, which will be useful if proved correct. It is unlikely to be more accurate than when applied to non-electrolytes (see p. 348).

The Effect of pH on Membrane Permeability to Ions. One of the most valuable variables in the study of ion permeability is pH; changes in this variable have been the subject of many extensive studies, some of which have been mentioned in the experimental chapters. The essential theory for interpretation of results has been given by Osterhout (1933) and Davson & Danielli (1936). Penetration of membranes by ions may be separated into three types:

- (1) The ion passes through a homogeneous lipid layer by simple diffusion.
- (2) The ion combines with an ion of opposite sign forming part of the membrane, and the resultant ionic doublet diffuses through the membrane.
- (3) The ion penetrates through pores.

In all three cases the sign and magnitude of the charge on the membrane are of importance in determining the rate of penetration, and both sign and magnitude of the charge are changed by pH changes. The charge density at the surface of cells varies between 200 and 25,000 e.s.u. per cm.², or at most one CO₂⁻ group per 200 sq. Å.; usually the area per charge is much greater.* But from other considerations (Harvey & Danielli, 1938) we know that the surface is largely composed of polar groups, and can hardly have less than four groups per 200 sq. Å. These other groups must be the OH groups of sterols, lipin groups, and the CO.NH groups of proteins, etc. All of these groups contain electrostatic charges which are oriented at the oil-water interface, mostly with the negative ends of the dipoles turned towards the water phase, and the positive ends turned towards the oil phase.

* Calculated from data given by Abramson, *Electrokinetic Phenomena*, 1934.

Thus, due to the polar groups, an electric field arises as shown in fig. 71 *a*.

When ions are present they will tend to accumulate round the ends of the dipoles of opposite sign, producing the modified electrostatic potential diagram of Fig. 71 *b*.^{*} We see that when a dipole has its positive end in the oil (or membrane) phase,

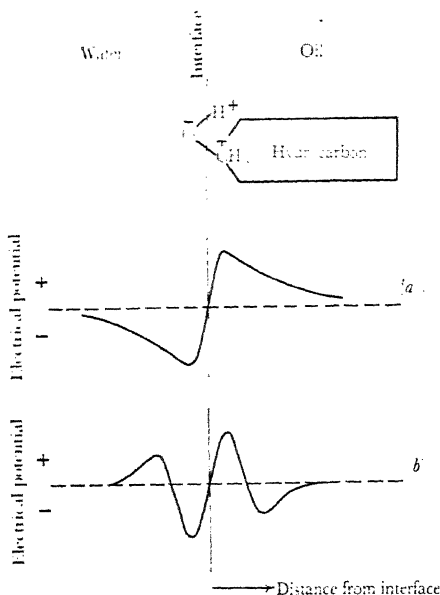


FIG. 71. The electrical field at an oil-water surface: *a*) due to the dipoles oriented at the surface, *b*) as modified by the presence of salt.

there will be an excess of negative ions in the oil (or membrane) phase, adjacent to the dipole. Davson & Danielli (1936) pointed out that, with all the compounds likely to occur in the cell membrane, the dipoles are in fact oriented with their positive ends in the oil phase over the pH range 5–8, and for the great majority of compounds (all except fatty acids) this is true over the pH range 5–13. Consequently, it is highly improbable that the direction of

^{*} This conception has also been used by Gatty & Dean (1940) to explain the nature of surface potentials of monolayers at the air-water interface.

the electrostatic field due to the oriented dipoles will change its sign over the physiological pH range. *Hence any action of this electrostatic field on ion permeability will also show no change in sign over the physiological pH range.*

Now consider the potential energy diagram presented by such a membrane, in which the two interfaces are so close that the two regions of excess negative charge inside the membrane overlap. As we have seen in the earlier part of this chapter even a molecule without a fixed net charge meets a system of potential energy barriers, which may be represented in a smoothed-out form by Fig. 72(a). Then, if charged, the ion will meet additional barriers, represented by Fig. 72(b) for a positively charged ion, and Fig. 72(c) for a negatively charged ion. If we sum these two potential energy terms we obtain the two curves of Fig. 72(d), which show us that, for a sufficiently narrow membrane, the potential energy of negative ions inside the membrane is less than the potential energy of positive ions, i.e. the oil-water partition is greater for negative ions than for positive ions of the same structure. As the thickness of the membrane is increased this effect will fall off rapidly. Thus, for a sufficiently thin membrane, since the partition coefficient of negative ions is greater than that of positive ions, the permeability to negative ions will be greater than that to positive ions.

Hence we conclude that, for simple diffusion of ions of similar structure through a lipid membrane, (1) there will be no reversal of selective permeability over the physiological pH range, (2) if there is a selective activity, negative ions will penetrate more rapidly than positive ions. This may be the cause of the selective permeability of red cells to negative ions.

Where penetration is preceded by complex formation, the formation of complexes can be detected by the variation in permeability with concentration of the penetrating ion (Osterhout, 1933; see also p. 198). Obviously complex formation can only occur when the membrane contains a lipid-soluble anion, if cations are transported, or lipid-soluble cations, if anions are transported. If both lipid-soluble anions and cations are present, there will be an excess of the anions on the alkaline side of the iso-electric point, and an excess of cations on the acid side. Hence the selectivity of the membrane to ions of similar structure will change in the region of the iso-electric point. If only either

lipoid-soluble cations or anions are present, the pH at which selectivity falls off will depend upon the strength of the basic or acidic groups of the ions concerned.

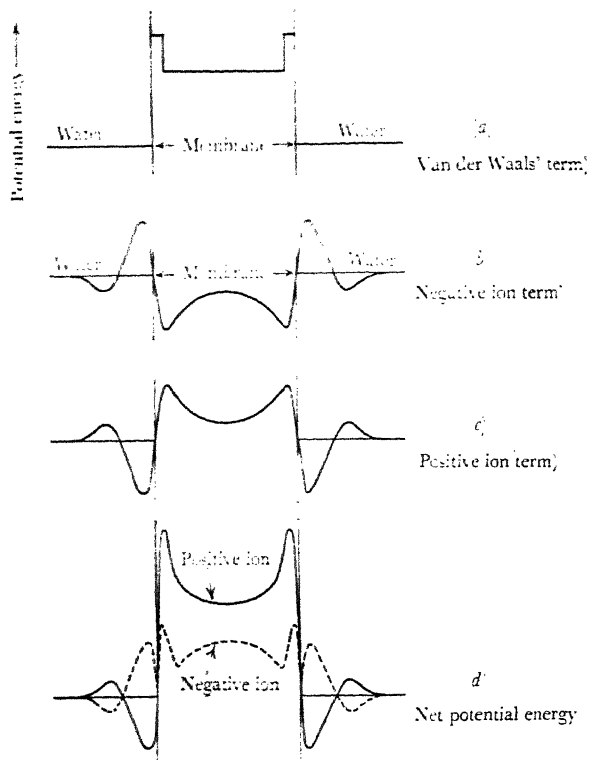


FIG. 72. Potential energy barrier diagrams of the cell membrane: (a) met by uncharged molecule, (b) extra term due to negative charge, (c) extra term due to positive charge, (d) summed potential energy $(a+b)$ for negative ion and $a+c$ for positive ion.

In the case of red cells, there is normally a negligible permeability to cations, and a moderately large permeability to anions, the latter apparently due to simple diffusion. In other cells there is a moderate permeability to anions, and a variable, sometimes rather large, permeability to cations. In some cases where cation permeability is large and non-pathological, complex-

formation in the membrane appears to occur—this view has been particularly advanced by Osterhout and his colleagues.

With the third type of penetration—through pores—Perrin pointed out that the selective action must (and does) reverse in sign at the iso-electric point. Davson & Danielli (1936) have shown that the selectivity will change at the iso-electric point, whether this point is fixed by ionic charges of membrane molecules, or by the polar group charges of membrane molecules.

It is therefore theoretically possible to distinguish between the three possible modes of penetration of the cell membrane by the following steps: (1) study of the influence of concentration; this will pick out complex formation: (2) study of the effect of pH in other cases; reversal of selectivity at the iso-electric point means a pore-penetrating mechanism. If there is no reversal in selectivity, then the mechanism probably involves simple penetration of a lipid layer.

There is a fourth point, the variation of the membrane structure with pH which, unfortunately, may easily obscure influences by the other factors already enumerated. It is possible that the increase in cation permeability with alkalinity in the case of the human erythrocyte in isotonic sucrose (Maizels, 1935; Davson, 1939), of the cat erythrocyte in isotonic KCl (Davson, 1940), and the increase in anion permeability of the rabbit erythrocyte (Parpart, 1940), are all instances of this sort of influence (see Chapter XII).

Effect of Diffusion Potentials. Diffusion potentials arise when one or more types of ion in a system diffuses faster than other types. Consider the simple case of a Donnan equilibrium, in which in solution A there are fixed anions unable to diffuse through a membrane into a solution B . Then at equilibrium if the volume of A is fixed the concentration of cations in A will be greater than the concentration in B . Let B contain $0.1 M Ca^{++}$ and $0.1 M Na^+$, and the equilibrium concentration of Na^+ in A be $1.0 M$. Then

$$\frac{[Na]_A}{[Na]_B} = \frac{\sqrt{[Ca]_A}}{\sqrt{[Ca]_B}} = \text{constant.}$$

$$\therefore \frac{1}{0.1} = \frac{\sqrt{[Ca]_A}}{\sqrt{0.1}} \quad \text{or} \quad [Ca]_A = 3.3 M.$$

Thus, in this particular case, the concentration of sodium in A is 10 times greater than in B , and the concentration of calcium is

33 times greater. Since the equilibrium is a dynamic one, i.e. ions are continually passing from $B \rightarrow A$ and from $A \rightarrow B$, we can write:

$$\begin{aligned} & \text{[concentration of sodium in } A \times \text{rate of diffusion } A \rightarrow B] \\ &= \text{[concentration of sodium in } B \times \text{rate of diffusion } B \rightarrow A] \end{aligned}$$

or

$$C_A P_{AB} = C_B P_{BA}.$$

Hence $1.0 \times P_{AB} = 0.1 \times P_{BA}$, i.e. $P_{BA} = 10 P_{AB}$.

Similarly, for calcium, $P_{BA} = 33 P_{AB}$.

In other words, the inability of certain anions in A to diffuse into B has made the apparent average rate of diffusion, from $B \rightarrow A$, 10 times greater than from $A \rightarrow B$, in the case of sodium ions, and 33 times greater with calcium ions. How does this arise?

The condition for equilibrium is that the sodium concentration in A shall be 10 times that in B , and the factor determining this unequal ratio is the presence of indiffusible ions in A . These ions can only exert their influence on the diffusion of Na^+ ions by virtue of their electrostatic charge. There is a tendency for sodium ions to escape from A to B , but the escaping Na^+ ions leave behind them negative charges, so that a potential difference is finally built up sufficient to reduce the permeability P_{AB} and raise the permeability P_{BA} until $C_A P_{AB} = C_B P_{BA}$. Thus, in this case, an inability of certain ions to diffuse causes not only an unequal equilibrium distribution of all diffusible ions, but also, by setting up a diffusion potential, changes the actual values of the observed permeability constants—increasing P_{BA} and decreasing P_{AB} , which would otherwise be equal. Similarly, diffusion potentials due to other circumstances modify ion penetration rates, and it is not necessary for this effect that an ion shall have zero mobility, as in the case of the Donnan equilibrium: it is only necessary that the intrinsic diffusion constants of the ions shall differ. In attempting to deal quantitatively with ion permeability, it is therefore necessary to be able to deal with the terms due to diffusion potentials.

Effect of Partition Coefficients, Pore Size, Ion Diameter, Hydration, etc.

When penetration of an ion occurs through a pore, ion diameter and pore diameter are convenient variables to consider, if only qualitative or semi-quantitative theoretical discussion is possible. For quantitative treatment it is necessary to know the values of the activation energy of diffusion, or to have some other equivalent

measurement, but at present this is hardly possible. Discussion of the pore or sieve effect for ions is given in Chapter xv; the theory of Meyer & Sievers also takes account of variation in partition coefficient, but is not applicable to very thin membranes.

In experiments on cell membranes the series of ions arranged in order of ionic diameter is subject to unknown effects due to variations in partition coefficients, and also the exact diameter of an ion is difficult to determine, since it is affected by hydration. The degree of hydration of ions is known to follow the series $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$ (the lyotropic series), and where such a series is encountered in cell permeability it is plausible to attribute the series to the effect of ionic diameter—but it is equally plausible to attribute it to variations in partition coefficients. Quantitative work, essential for achieving a distinction between these two factors, is hampered by the fact that the amount of water of hydration of a given ion is indefinite, and varies within wide limits according to the method of measurement. Until such factors can be dealt with it is impossible to make a satisfactory analysis of permeability to ions, or of the effect of narcotics on ion permeability.

In conclusion, it may be said that the theory of penetration of non-electrolytes is now taking a very definite shape, and we can deal in at least a semi-quantitative way with all the main variables concerned. But the complicating effect of the electrostatic charge of ions has so far prevented a similar result being achieved for ions. The problem of special permeability to particular substances is also approaching a solution, in the achievement of which the studies of Schulman, Rideal and their colleagues on molecular interactions in monolayers may well prove to be the spearhead.

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APPENDIX A

THE THEORY OF PENETRATION OF A THIN MEMBRANE

By J. F. DANIELLI

DEDUCTION OF A FUNDAMENTAL EQUATION

THE plasma membrane of most cells is, to a first approximation, a thin layer of lipoid not more than 10^{-6} cm. in thickness. In the following matter discussion will be restricted to such thin layers of lipoid, though many of the conclusions reached are valid for membranes of other materials.

To a penetrating molecule a membrane presents three sites of resistance: (1) the membrane interface, for diffusion in the direction water \rightarrow lipoid; (2) the membrane interface, for diffusion in the direction lipoid \rightarrow water; (3) the interior of the membrane. All previous studies have assumed that (1) and (2) are so much less important than (3) that (1) and (2) may be neglected except in so far as they enter into partition coefficients. This is not a justifiable assumption, for such thin membranes.

The gross rate of passage of molecules from a solution of concentration C into the interior of the membrane is

$$\frac{dN}{dt} = aC, \quad \dots\dots (42)$$

and in the reverse direction, from the membrane into water,

$$\frac{dN'}{dt} = aC', \quad \dots\dots (43)$$

where C' is the concentration in the membrane. a and b are constants varying for different substances, and with temperature. These two equations define the rate of diffusion across the membrane interface. We now need to deal with the interior of the membrane.

One method is to use Fick's equation:

$$\frac{\partial S}{\partial t} = -D \frac{\partial C}{\partial x}, \quad \dots\dots (19)$$

where ∂S is the amount of substance diffusing across an area of 1 cm.² in time ∂t under a concentration gradient $\partial C/\partial x$. D is a

constant for a given medium, substance and temperature. With the three constants a , b and D , we can obtain an expression for the permeability P of the membrane in gram moles crossing unit area per sec. per gram mol. per litre concentration difference. Mr F.J. Turton has made such an analysis, finding that, if

$$\frac{bl_2}{D} \ll 1 \quad \text{and} \quad \frac{dS}{dt} = P(C - C_i),$$

then
$$\frac{C - C_i}{C} = \frac{m_2}{m_1 - m_2} e^{m_1 t} - \frac{m_1}{m_1 - m_2} e^{m_2 t}, \quad \dots\dots(44)$$

where
$$m_1 = \frac{l_1}{l_2} \left[-\frac{al_2}{2bl_3} - \frac{1}{2} \left(\frac{al_2}{2bl_3} \right)^2 \dots \right],$$

$$m_2 = \frac{b}{l_2} \left[-2 - \frac{al_2}{2bl_3} - \frac{1}{2} \left(\frac{al_2}{2bl_3} \right)^2 \dots \right],$$

C_i = concentration of the molecular species under consideration inside a cell of constant volume,

C = concentration outside the cell,

l_2 = thickness of the cell membrane,

l_3 = "equivalent thickness" of the cell, i.e. volume per sq. cm. of membrane.

This result (equation (44)) is valid within the range of values of a , b and D possible in cases where the rate of permeation can be observed experimentally, provided diffusion through the membrane is so much slower than through the aqueous phase that the membrane may be regarded as constituting the only significant barrier to diffusion. If, in addition,

$$\frac{2bt}{l_2} \gg 1 \quad \text{and} \quad \frac{al_2}{bl_3} \ll 1,$$

$$P = \frac{a}{2}. \quad \dots\dots(28)$$

In this case diffusion through the interior of the membrane, and from membrane into water, is so much faster than diffusion from water into membrane, that only the latter factor is of importance.

Unfortunately, Fick's equation is not necessarily applicable to such thin layers. Fick (1855) assumed that the resistance to diffusion of a solute molecule was due to a medium of particle size negligible compared with that of a diffusing molecule. But

in fact in the cell membrane the resistance to diffusion is due to large molecules such as cholesterol, much larger than most penetrating molecules. In a medium of molecules of the same or

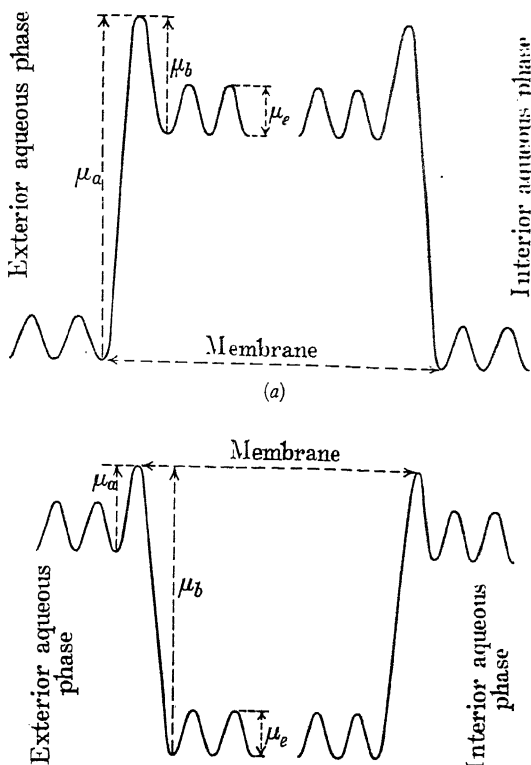


FIG. 73. Potential energy diagrams of the cell membrane: the potential energy barriers met (a) by a molecule such as glycerol, (b) by a molecule such as benzene or propane.

greater size than the solute molecule, the diffusing solute molecule does not encounter a smooth continuous resistance, but an intermittent resistance, which may be represented to a first approximation by the potential energy diagrams of Fig. 73. In this medium not every molecule will be diffusing simultaneously.

Many molecules will be vibrating about a mean position between adjacent potential energy barriers, and only those having more than the minimum kinetic energy μ_e necessary to pass over a potential energy barrier will in fact be capable of doing more than vibrate. The rate of diffusion through such a medium must now be considered.

Let the distance between adjacent potential energy minima be λ . Assume that the height of the potential energy maxima and minima does not vary with time, and that the diffusing molecules diffuse only when possessed of the minimum kinetic energy μ_e , necessary to break the bonds restricting diffusion.* Consider diffusion through a section of the medium 1 cm.² in area, containing n maxima. Let the concentration at one side of the section be kept at C_0 , at the other side at C_n , and the concentrations in successive minima be C_1, C_2, C_3, \dots etc. When the condition of steady flow is reached the concentration across each maximum will be the same, $\partial S / \partial t$. The rate of flow from C_0 to C_1 is

$$\frac{dN_0}{dt} = eC_0, \quad \dots\dots(45)$$

where e is a constant and is a function of μ_e . Similarly, the flow from C_1 to C_0 is

$$\frac{dN_1}{dt} = eC_1. \quad \dots$$

Hence the net flow from C_0 to C_1 is

$$\begin{aligned} \frac{dN_0}{dt} - \frac{dN_1}{dt} &= \frac{dS}{dt} = e(C_0 - C_1) \\ &= e(C_1 - C_2) = \dots = e(C_{n-1} - C_n) \\ &= -\frac{e}{n}(C_n - C_0). \end{aligned} \quad \dots\dots(47)$$

But Fick's equation for the state of steady flow is

$$\frac{dS}{dt} = -D \frac{C_n - C_0}{\lambda n}. \quad \dots\dots(19.1)$$

Combining (19.1) and (47):

$$D = \frac{n+1}{n} \lambda e. \quad \dots\dots(48)$$

* Due to Van der Waals' forces, hydrogen bonds, etc.

The expression $D = \{(n+1)/n\} \lambda e$ reduces to λe if n is large. But if the membrane is thin, n is small, and D is a function of n , i.e. of the membrane thickness. With this established we may now proceed to deal with the membrane as a whole.

To different molecules the cell membrane will present different potential energy barriers, e.g. Fig. 73 (a) (for a polar molecule such as glycerol $\text{CH}_2\text{OH} \cdot \text{CHOH} \cdot \text{CH}_2\text{OH}$); Fig. 73 (b) (for a non-polar molecule such as propane $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_3$). But in all cases there are three types of barrier, one μ_a met on entering the membrane, one μ_b met on leaving the membrane, and n of height μ_c met in the interior of the membrane.

Consider the case where the concentration on the two sides is kept steady at C and C_i respectively. When the condition of steady flow is reached, the rate of flow across every barrier is the same, dS/dt . From the minor barriers we obtain equation (47). For the major barriers

$$\begin{aligned} \frac{dS}{dt} &= aC - bC_0 = bC_n - aC_i \\ &= \frac{a}{2} (C - C_i) - \frac{b}{2} (C_0 - C_n). \end{aligned} \quad \text{..... (49)}$$

Combining (47) and (49):

$$C_0 - C_n = \frac{a}{b + 2e/n} (C - C_i). \quad \text{..... (50)}$$

Substituting for $(C_0 - C_n)$ in (47) from (50):

$$\frac{dS}{dt} = \frac{e}{n} \cdot \frac{a}{b + 2e/n} (C - C_i). \quad \text{..... (51)}$$

But

$$\frac{dS}{dt} = P(C - C_i).$$

$$\therefore P = \frac{ae}{nb + 2e}. \quad \text{..... (26)*}$$

This is the fundamental equation defining the permeability of a homogeneous lipid membrane.

The equation $P = ae/(nb + 2e)$ has been obtained for a homogeneous membrane. For an inhomogeneous membrane we must

* I am greatly indebted to Mr F.J. Turton for this simple analysis.

treat each area separately, i.e. obtain a term $a_r e_r (n_r b_r + 2e_r)$ for the r th homogeneous element and then sum, obtaining

$$P = \sum \frac{ae}{nb + 2e}.$$

The deduction of the equation $P = ae (nb + 2e)$ is based on a few simple assumptions about the structure of matter. In its general form it is undoubtedly correct, and further advance in this matter must consist in filling in the details of our knowledge of the way in which a , b , e and n vary with molecular species, time, temperature, etc.

Special Cases

Consider a homogeneous lipoid membrane to which the equation $P = ae (nb + 2e)$ applies. The value of μ_b is greater than μ_e for the great majority of substances which penetrate cell membranes very slowly; hence for such substances b is much less than e , and with thin membranes, i.e. when n is small, nb may be neglected compared with $2e$. Hence

$$P = \frac{a}{2}, \quad \dots\dots(28)$$

i.e. for molecules which penetrate a thin membrane very slowly, the rate of passage over the oil-water interface in the direction water \rightarrow oil is often the term dominating the rate of penetration. This is the same result as was obtained by Turton, assuming that Fick's equation could be applied to thin membranes. The reason why the same result is obtained from both sets of assumptions is that the effective resistance to free diffusion is almost entirely located at the oil-water interface, so that the nature of diffusion in the interior of the membrane is, in this case, unimportant.

On the other hand, for many very rapidly penetrating molecules nb is equal to, or often greater than, $2e$. In this latter case we obtain

$$P = \frac{a}{b} \cdot \frac{e}{n}. \quad \dots\dots(27)$$

Thus we obtain simplified versions of equation (26) for very slowly and for very rapidly penetrating molecules.

An Approximate Method for Evaluating a , b , e and n (1) *Theoretical Aspects.*

There is no accurate method yet available for evaluating a , b and e . The following procedure yields an approximate result.

Consider equation (42), $dN/dt = eC_0$, which gives the rate of flow of molecules from one side to the other of a single potential energy barrier of height μ_e calories. If the media on either side of the barrier were perfect gases, e would be given by

$$e = \sqrt{\frac{RT}{2\pi M}} e^{-\frac{\mu_e}{RT}}, \quad \dots\dots(52)$$

where R is the gas constant per gram mol., T is absolute temperature, M is molecular weight. If the medium is not a perfect gas, the numerical coefficient of the terms on the right will be different, and we shall multiply the right-hand side by a constant r to compensate for this. This correction is purely empirical. Then, in addition, not every molecule having the minimum kinetic energy μ_e will in fact be able to diffuse; let ϕ_e be the probability that a molecule having the activation energy μ_e will actually diffuse across a potential energy barrier. Then

$$e = r\phi_e \sqrt{\frac{RT}{2\pi M}} e^{-\frac{\mu_e}{RT}} \quad \dots\dots(53)$$

$$\text{and} \quad (Q_{10})_e = \frac{e_{T+10}}{e_T} = \sqrt{\frac{T+10}{T}} e^{\frac{\mu_e}{RT} \left(\frac{10}{T-10} \right)} \quad \dots\dots(54)$$

This semi-empirical equation needs testing to ascertain its limits of accuracy. This can be done in the following ways: (1) keeping M constant and varying the temperature: then any failure in e to follow the predicted course must be due to variation in $r\phi_e$, or some inadequacy in the terms $\sqrt{T}e^{-\mu_e/RT}$. A suitable method for this study is the rate of diffusion of molecules across the liquid-vapour interface at various temperatures; it is found that, if allowance is made for the variation in μ_e with temperature, the terms $\sqrt{T}e^{-\mu_e/RT}$ are accurate, to a first approximation, and the value of e at any temperature may be calculated from the value at any other temperature within a range of 50° C. with an accuracy of $\pm 10\%$. (2) Keep the temperature constant, and vary M . This tests the terms $\sqrt{(1/M)} e^{-\mu_e/RT}$. This may be done

using the Fick diffusion constant for diffusion in liquids, for, as we saw above, $e = D \lambda$, so that

$$D = \lambda r \phi \sqrt{\frac{RT}{2\pi M}} e^{-\frac{\mu_e}{RT}}, \quad \text{.....(55)}$$

and at constant T :
$$\frac{DM^{\frac{1}{2}}e^{\mu_e}}{\lambda} = \text{constant.} \quad \text{.....(56)}$$

Or, since λ is approximately constant also, $DM^{\frac{1}{2}}e^{\mu_e}$ should be a constant, say β , if the terms $M^{\frac{1}{2}}e^{\mu_e}$ in equation (53) are correct. Unfortunately there are very few accurate values for μ_e under these conditions, but with the values available we can show that from the average value of β , the value of D for any other molecule up to the size of glucose can be calculated, with an error not greater than $\pm 100\%$ in solvents such as water and alcohol.

The validity of Thoevert's equation, $DM^{\frac{1}{2}} = \text{constant}$ (see Chapter v), is probably due to the fact that e^{μ_e} does not vary greatly in solvents of low viscosity, so that from (56) we have $DM^{\frac{1}{2}} = \text{constant}$, approximately.

Summing up, equation (53) must be regarded as approximate only, but it can be used for approximate work. The error involved in the calculation of e by this equation is probably much less than a factor of fivefold, so that if we can assume the calculated value of e may be five times less or five times greater than the true value, we shall probably be within safe limits.

This leaves the terms a , b and n . Of these a and b will be of the same form as e . n cannot be estimated accurately, but cannot be less than 10^{-8} cm. Hence if the thickness of a membrane is divided by 10^{-8} we obtain a *maximum* value of $n=50$, in the case of a membrane 50 Å. thick. The relative values of a and b can also be obtained from the partition coefficient B , for

$$B = \frac{a}{b}. \quad \text{.....(57)}$$

Now consider the special case of equation (28). Substituting from (53) we have

$$P = \frac{a}{2} = \frac{1}{2} r \phi_a \sqrt{\frac{RT}{2\pi M}} e^{-\frac{\mu_a}{RT}}. \quad \text{.....(58)}$$

Hence
$$(Q_{10})_P = \sqrt{\frac{T+10}{T}} e^{\frac{\mu_a}{RT} \left(\frac{10}{T+10} \right)}. \quad \text{.....(59)}$$

And substituting for $e^{\mu_a/RT}$ in (57):

$$PM^{\frac{1}{2}} Q_{10}^{(T+10)/10} = r\phi_a \sqrt{\frac{RT}{8\Pi}} \left(\sqrt{\frac{T+10}{T}} \right)^{\frac{T-10}{10}} \dots\dots 60.$$

= constant, at constant temperature.

Similarly, in the special case of equation (27):

$$P = \frac{a}{b} \cdot \frac{e}{n} = \frac{r\phi_a\phi_e}{n\phi_b} \sqrt{\frac{RT}{2\Pi M}} e^{\frac{\mu_b - \mu_a - \mu_e}{RT}} \dots\dots 61.$$

Hence $(Q_{10})_P = \sqrt{\frac{T+10}{T}} e^{-\frac{\mu_b - \mu_a - \mu_e}{RT}} \left(\frac{10}{T+10} \right)^{\frac{10}{T+10}} \dots\dots 62.$

And $PM^{\frac{1}{2}} Q_{10}^{(T+10)/10} = \frac{r\phi_a\phi_e}{n\phi_b} \sqrt{\frac{RT}{2\Pi}} \left(\sqrt{\frac{T+10}{T}} \right)^{\frac{T-10}{10}} \dots\dots 63.$

= constant, at constant temperature.

Probably $\phi_a \approx \phi_b \approx \phi_e$, so that ϕ_e and ϕ_b in equations (61) and (63) cancel out.

So we find that, if equation (53) is approximately correct, then for the two special cases of equations (27) and (28) $PM^{\frac{1}{2}} Q_{10}^{(T+10)/10}$ is constant at constant temperature, and the temperature variation of P may be calculated. $PM^{\frac{1}{2}}$ and Q_{10} are negatively correlated. For a membrane made up of areas of varying properties, Q_{10} and P will differ from place to place. If each area were treated separately, we should find $\Sigma PM^{\frac{1}{2}} Q_{10}^{(T+10)/10} = \text{constant}$. But experimentally we measure an *average* Q_{10} and an *average* P , and hence for such a membrane $PM^{\frac{1}{2}} Q_{10}^{(T+10)/10}$ will not be constant.

For homogeneous membranes, owing to the deficiencies in equation (53), permissible variations in $PM^{\frac{1}{2}} Q_{10}^{(T+10)/10}$ will lie between values five times less and five times greater than the average value.

Since $PM^{\frac{1}{2}}$ and (Q_{10}) are negatively correlated for a homogeneous membrane, if this negative correlation is not observed in a particular series of experiments, the membrane involved cannot be homogeneous. This applies to all types of molecules, not only those obeying (27) and (28).

(2) *Practical Aspects.*

In practice one can make only two direct observations, giving (1) the permeability constant P ; (2) the Q_{10} of P . Hence to obtain values of a , b , e and n we must resort to a number of devices. In

the first place we shall not be able to make the fullest quantitative use of equation $P = ae^{(nb + 2e)}$, but shall be restricted for quantitative work to the special case of slowly penetrating molecules obeying the equation $P = a/2$. To prove that this equation is obeyed we must prove that $nb \ll 2e$, i.e. we must know the minimum value of e , and the maximum value of n and b .

TABLE LXXI

All figures here refer to flow per sq. cm. per sec. per gram mol. per c.c. concentration.

Substance	D_{app}	Min. value of $2e$	η	Min. value of μ	Max. value of a	B	Max. value of nb ($=50a/B$)
Propyl alcohol	9.3×10^{-3}	3.72	1.37	5.100	1.22×10^5	0.005*	1.22×10^3
Urea	9.4×10^{-3}	3.76	1.86	10,200	1.81×10^4	0.05	6.0×10^5
Thiourea	8.25×10^{-3}	3.3	2.14	12,750	2.9×10^{-1}	0.015*	6.7×10^2
Glycol	9.3×10^{-3}	3.72	2.92	18,400	1.41×10^{-3}	0.00049	1.44
xy Dioxy- propane	8.25×10^{-3}	3.3	3.75	22,500	1.03×10^{-3}	0.0059	8.7×10^{-5}
Glycerol	7.2×10^{-3}	2.88	3.65	22,200	1.62×10^{-8}	0.00007	1.15×10^{-2}
xy Dioxy- propane	8.25×10^{-3}	3.3	3.31	20,300	4.28×10^{-7}	0.001	2.14×10^{-2}
Diethylene glycol	6.9×10^{-3}	2.76	3.42	20,800	1.26×10^{-7}	0.005*	1.26×10^{-3}
Triethylene glycol	5.75×10^{-3}	2.3	3.34	20,500	2.22×10^{-7}	0.02*	5.55×10^{-4}

* Approximate values.

The interior of the cell membrane is a hydrocarbon liquid and its viscosity cannot be more than 10^4 to 10^5 times greater than that of water—if the membrane were of olive oil, for example, the viscosity would be only 10^2 times greater than water. Hence the minimum value of the Fick diffusion constant D in the membrane interior cannot be less than about 10^{-4} of the value in water D_w . Hence

$$e = \frac{D}{\lambda} = \frac{D_{\text{water}}}{\lambda} \times 10^{-4}.$$

The maximum possible value of λ is 5×10^{-7} cm. (it is probably nearer to 10^{-8} cm.). Hence the *minimum* possible value of e is given by

$$e = \frac{10^{-4} D_{\text{water}}}{5 \times 10^{-7}} = 2 \times 10^2 D_w. \quad \dots\dots (64)$$

The *maximum* possible value of n , for a membrane about 50 Å. thick, has been given above as 50.

$$\text{Lastly, } b \text{ is given by } B = \frac{a}{b}, \quad \dots\dots (57)$$

where B is the oil-water partition coefficient. Hence to find b we need a , which must be obtained from equation (53). Now in the equation $P = ae/(nb + 2e)$ the term having the largest Q_{10} is a . Hence the Q_{10} of P is either equal to the Q_{10} of a , if the molecular species obeys the simplified form $P = a/2$, or else is less than the Q_{10} of a , i.e. the Q_{10} of $P \leq Q_{10}$ of a . The higher the Q_{10} of a , the smaller is a . Hence, if we calculate $e^{\mu_a/RT}$ from (59), we shall obtain the maximum possible value of a when this value of $e^{\mu_a/RT}$ is substituted in equation (58). The term $\sqrt{RT/2\pi M}$ of 58 is also known, so that now we only need the value of $r\phi_a$ to get the maximum possible value of a . In solvents such as water, benzene and alcohol, $r\phi_a$ varies very little, and is about 10^5 to 10^6 . In solvents consisting of larger molecules, $r\phi$ is probably smaller, so that if we use the value 10^5 we have the maximum possible value of $r\phi$. Hence the maximum possible value of a is

$$a = 10^5 \sqrt{\frac{RT}{2\pi M}} e^{-\frac{\mu_a}{RT}} \text{ per cm.}^2 \text{ per sec. per mol. per litre,} \\ \dots\dots\dots 65$$

where $e^{\mu_a/RT}$ is calculated from the Q_{10} of P . Then the maximum value of b can be obtained from equation (57).

TABLE LXXII

Substance	P	Q_{10}	$PM^{\frac{1}{2}}Q_{10}^{\frac{1}{2}}e^{-\frac{\mu_a}{RT}}$
Propyl alcohol	10.6×10^{-16}	1.37	1.1×10^{-10}
Urea	7.8×10^{-16}	1.86	8.5×10^{-7}
Thiourea	0.019×10^{-16}	2.14	1.7×10^{-7}
Glycol	0.209×10^{-16}	2.92	2.06×10^{-2}
$\alpha\beta$ Dioxyp propane	0.405×10^{-16}	3.75	82.0
Glycerol	0.0017×10^{-16}	3.65	0.17
$\alpha\gamma$ Dioxyp propane	0.105×10^{-16}	3.31	0.48
Diethylene glycol	0.075×10^{-16}	3.42	1.16
Triethylene glycol	0.0333×10^{-16}	3.34	0.28

We now have the minimum value of e , maximum value n and b . If $nb \ll 2e$, then if the membrane is homogeneous $PM^{\frac{1}{2}}Q_{10}^{(T+10)/10} = \text{constant}$. If this quantity is not constant for molecules for which $nb \ll 2e$, the membrane is not homogeneous. Tables LXXI and LXXII show an example of this treatment applied to the data of Jacobs *et al.* (1935) for ox red cells, assuming the partition coefficient membrane/water = partition coefficient olive oil/water. It will be seen that only $\alpha\beta$ dioxyp propane, $\alpha\gamma$ dioxyp propane, glycerol, diethylene glycol and triethylene glycol have maximum values of $nb \ll 2e$. Of these molecules $\alpha\beta$

dioxypropane is unsymmetrical and will not have the same ϕ value as the other molecules. This leaves the last four molecules, which should obey the equation $P=a/2$, so that if the membrane is homogeneous $PM^{\frac{1}{2}}Q_{10}^{T-10/10}$ should be approximately constant. Table LXXII shows that this quantity is within the permissible limits of variation. Hence the membrane must be homogeneous, to a first approximation.

The Quantitative Relationships between Permeability and Partition Coefficients. In the case of very slowly penetrating molecules for which $P=a/2$, we can use (57) to relate P to the partition coefficient B : then

$$P = \frac{a}{2} = \frac{1}{2}bB$$

$$= \frac{1}{2}B\tau\phi_b \sqrt{\frac{RT}{2\pi M}} e^{-\frac{\mu_b}{RT}}$$

or $PM^{\frac{1}{2}}e^{\frac{\mu_b}{RT}} = B \times \text{constant, at constant temperature.}$
.....(66)

Hence $PM^{\frac{1}{2}}e^{\mu_b/RT}$ is a linear function of the partition coefficient B . μ_b is given approximately by $\mu_b = 750x$ calories, where x = number of unscreened CH_2 groups per molecule. CH_2 groups screened from the solvent by polar groups such as OH do not contribute to x .

In the other extreme case, very rapidly penetrating molecules,

$$P = \frac{a}{b} \cdot \frac{e}{n} = B \times \frac{e}{n}$$

$$= \frac{B}{n} \tau\phi_c \sqrt{\frac{RT}{2\pi M}} e^{-\frac{\mu_c}{RT}}.$$

Or, since μ_c probably varies only slightly from substance to substance in liquids,

$PM^{\frac{1}{2}} = B \times \text{constant, at constant temperature.}$ (67)

Hence in this case $PM^{\frac{1}{2}}$ is, to a first approximation, a linear function of the partition coefficient. Both these relationships (66) and (67) are subject to the accuracy limits of a factor of fivefold mentioned earlier in connection with equation (53).

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